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(71) Applicant: AKTIESELSKABET DE DANSKE
 SUKKERFABRIKKER, Langebrogade 5,
 DK-1411 Copenhagen K (DK)

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(72) Inventor: Marcker, Kjeld Adrian, 1, Toftevej, DK-8250 Ega
 (DK)
 inventor: Jensen, Jens Stougaard, 10, J.M. Mørksgade 1.
 sel, DK-8000 Aarhus C (DK)

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(74) Representative: Vossius & Partner,
 Siebertstrasse 4 P.O. Box 86 07 67,
 D-8000 München 86 (DE)

(54) Method for the expression of genes in plants.

(57) A method for the expression of genes in plants, parts of plants, and plant cell cultures, in which a DNA fragment is used comprising an Inducible plant promoter of root nodule-specific genes, DNA-fragments comprising an inducible plant promoter, to be used when carrying out the method, said DNA-fragments being identical with, derived from or comprising a 5' flanking region of root nodule-specific genes of any origin as well as plasmids and transformed Agrobacterium rhizogenes-strain which can be used when carrying out the method.

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A method for the expression of genes in plants,
parts of plants, and plant cell cultures, and DNA
fragments, plasmids, and transformed microorganisms
to be used when carrying out the method, as well
5 as the use thereof for the expression of genes in
plants, parts of plants, and plant cell cultures.

The invention relates to a novel method for the expression of genes in plants, parts of plants, and plant cell cultures, as well as DNA fragments 10 and plasmids comprising said DNA fragments to be used when carrying out the method. The invention furthermore relates to transformed plants, parts of plants and plant cells.

The invention relates to this method for the expression of genes of any origin under control of 15 an inducible, root nodule specific promoter.

The invention relates especially to this method for the expression of root nodule-specific genes in transformed plants including both leguminous 20 plants and other plants.

The invention relates furthermore to DNA fragments comprising an inducible plant promoter to be used when carrying out the method, as well as plasmids comprising said DNA fragments.

25 In the specification i.a. the following terms are used:

Root nodule-specific genes: Plant genes active only in the root nodules of leguminous plants, or

genes with an increased expression in root nodules. Root nodule-specific plant genes are expressed at predetermined stages of development and are activated in a coordinated manner during the symbiosis whereby a nitrogen fixation takes place and the fixed nitrogen is utilized in the metabolism of the plant.

Inducible plant promoter: Generally is meant a promoter-active 5' flanking region from plant genes inducible from a low activity to a high activity. In relation to the present invention "inducible plant promoter" means a promoter derived from, contained in or being identical with a 5' flanking region including a leader sequence of root nodule-specific genes and being capable of promoting and regulating the expression of a gene as characterised in relation to the present invention.

Leader sequence: Generally is meant a DNA sequence being transcribed into a mRNA, but not further translated into protein. The leader sequence comprises thus the DNA fragment from the start of the transcription to the ATG codon constituting the start of the translation. In relation to the present invention "leader sequence" means a short DNA fragment contained in the above inducible plant promoter and typically comprising 40-70 bp and which may comprise sequences being targets for a posttranscriptional regulation.

Promoter region: A DNA fragment containing a promoter which comprises target sequences for RNA polymerase as well as possible activation regions

comprising target sequences for transcriptional effector substances. In the present invention, target sequences for transcriptional effectors may also be situated 3' to the promoter, i.e. in the 5 coding sequences, the intervening sequences or on the 3' flanking region of a root nodule-specific gene.

Furthermore a number of molecular-biological terms generally known to persons skilled in the art are used, including the terms stated below:

CAP (addition) site: The nucleotide of the 5' end of the transcript where 7-methylGTP is added; In the Figures often given also as an asterisk *-marked nucleotide on a given nucleotide sequence.

15 DNA sequence or DNA segment: A linear array of nucleotides interconnected through phosphodiester bonds between the 3' and 5' carbon atoms of adjacent pentoses.

20 Expression: The process undergone by a structural gene to produce a polypeptide. It is a combination of transcription and translation as well as possible posttranslational modifications.

25 Flanking regions: DNA sequences surrounding coding regions. 5' flanking regions contain a promoter. 3' flanking regions may contain a transcriptional terminator etc.

Gene: A DNA sequence composed of three or four parts, viz. (1) the coding sequence for the gene

product, (2) the sequences in the promoter region which control whether or not the gene will be expressed, (3) those sequences in the 3' end conditioning the transcriptional termination and optionally polyadenylation, as well as (4) intervening sequences, if any.

Intervening sequences: DNA sequences within a gene which are not coding for any peptide fragment. The intervening sequences are transcribed into pre-mRNA and are eliminated by modification of pre-mRNA into mRNA. They are also called introns.

Chimeric gene: A gene composed of parts from various genes. E.g. the chimeric Lbc₃-5'-3'-CAT is composed of a chloroamphenicol acetyltransferase-coding sequence deriving from E. coli and 5' and 3' flanking regulatory regions of the Lbc₃ gene of soybean.

Cloning: The process of obtaining a population of organisms or DNA sequences deriving from one such organism or sequence by asexual reproduction, or more particular a process of isolating a particular organism or part thereof, and the propagation of this subfraction as a homogeneous population.

Coding sequences: DNA sequences determining the amino acid sequence of a polypeptide.

Cross-inoculation group: A group of leguminous plant species capable of producing functionally active root nodules with Rhizobium bacteria isolated from root nodules of other species of the group.

Leghemoglobin (Lb): An oxygen-binding protein exclusively synthesized in root nodules. The Lb proteins regulate the oxygen partial pressure in the root nodule tissue and transport oxygen to the 5 bacteroides. In this manner the oxygen-sensitive nitrogenase enzyme is protected. The Lb genes are root nodule-specific genes.

Messenger-RNA (mRNA): RNA molecule produced by transcription of a gene and possibly modification of 10 mRNA. The mRNA molecule mediates the genetic message determining the amino acid sequence of a polypeptide by part of the mRNA molecule being translated into said peptide.

Downstream: A position in a DNA sequence. It is 15 defined relative to the transcriptional direction 5'- 3' of the gene relative to which the position is stated. The 3' flanking region is thus positioned downstream of the gene.

Nucleotide: A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via a glycosidic bond (1' carbon of the pentose), and this combination of base and sugar is a nucleoside. The base characterises the nucleotide. The four DNA bases are 25 adenine (A), guanine (G), cytosine (C), and thymine (T). The four RNA bases are A, G, C, and uracil (U).

Upstream: A position in a DNA sequence. It is defined relative to the transcriptional direction 30 5'- 3' of the gene relative to which the position

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is stated. The 5' flanking region is thus positioned upstream of this gene.

5 Plant transformation: Processes leading to incorporation of genes in the genome of plant cells in such a manner that these genes are reliably inherited through mitosis and meiosis or in such a manner that these genes are only maintained for short periods.

10 Plasmid: An extra-chromosomal double-stranded DNA sequence comprising an intact replicon such that the plasmid is replicated in a host cell. When the plasmid is placed within a unicellular organism, the characteristics of that organism are changed or transformed as a result of the DNA of the plasmid. For instance a plasmid carrying the gene for tetracycline resistance (Tc^R) transforms a cell previously sensitive to tetracycline into one which is resistant to it. A cell transformed by a plasmid is called a transformant.

15 Polypeptide: A linear array of amino acids interconnected by means of peptide bonds between the α -amino and carboxy groups of adjacent amino acids.

Recombination: The creation of a new DNA molecule by combining DNA fragments of different origin.

20 Homologous recombination: A recombination between sequences showing a high degree of homology.

Replication: A process reproducing DNA molecules.

Replicon: A self-replicating genetic element possessing an origin for the initiation of DNA replication and genes specifying the functions necessary for a control and a replication thereof.

5 Restriction fragment: A DNA fragment resulting from double-stranded cleavage by an enzyme recognizing a specific target DNA sequence.

RNA polymerase: Enzyme effecting the transcription of DNA into RNA.

10 Root nodule: Specialized tissue resulting from infection of mainly roots of leguminous plants with Rhizobium bacteria. The tissue is produced by the host plant and comprises therefore plant cells whereas the Rhizobium bacteria upon infection are 15 surrounded by a plant cell membrane and differentiate into bacteroides. Root nodules are produced on other species of plants upon infection of nitrogen-fixing bacteria not belonging to the Rhizobium genus. Root nodule-specific plant genes are also 20 expressed in these nodules.

Southern-hybridization: Denatured DNA is transferred upon size separation in agarose gel to a nitro-cellulose membrane. Transferred DNA is analysed for a predetermined DNA sequence or a predetermined 25 gene by hybridization. This process allows a binding of single-stranded, radioactively marked DNA sequences (probes) to complementary single-stranded DNA sequences bound on the membrane. The position of DNA fragments on the membrane binding the probe 30 can subsequently be detected on an X-ray film.

Symbiotic nitrogen fixation: The relationship whereby bacteroides of root nodules convert the nitrogen (dinitrogen) of the air into ammonium utilized by the plant while the plant provides the bacteroides 5 with carbon compounds as a carbon source.

Symbiont: One part of a symbiotic relationship, and especially Rhizobium is called the microsymbiont.

Transformation: The process whereby a cell is incorporating a DNA molecule.

10 Translation: The process of producing a polypeptide from mRNA or:
the process whereby the genetic information present in a mRNA molecule directs the order of specific amino acids during the synthesis of a polypeptide.

15 Transcription: The method of synthesizing a complementary RNA sequence from a DNA sequence.

Vector: A plasmid, phage DNA or other DNA sequences capable of replication in a host cell and having one or a small number of endonuclease recognition 20 sites at which such DNA sequences may be cleaved in a determinable manner without loss of an essential biological function.

Traditional plant breeding is based on repeated crossbreeding of plant lines individually carrying 25 desired qualities. The identification of progeny lines carrying all the desired qualities is a particularly time-consuming process as the biochemical

and genetic basis of the qualities is usually unknown. New lines are therefore chosen according to their phenotype, usually after a screening of many lines in field experiments.

5 Through the ages a direct connection has existed between the state of nutrition, i.e. the health, of the population and the agricultural possibility of ensuring a sufficient supply of assimilable nitrogen in order to obtain satisfactory yields.

10 Already in the seventeenth century it was discovered that plants of the family leguminosae including beyond peas also beans, lupins, soybean, bird's-foot trefoil, vetches, alfalfa, sainfoin, and trefoil had an ability of improving crops grown on the habitat
15 of these plants. Today it is known that the latter is due to the fact that the members of the plants of the family leguminosae are able to produce nitrogen reserves themselves. On the roots they carry bacteria with which they live in symbiosis.

20 An infection of the roots of these leguminous plants with Rhizobium bacteria causes a formation of root nodules able to convert atmospheric nitrogen into bound nitrogen, which is a process called nitrogen fixation.

25 Atmospheric nitrogen is thereby converted into forms which can be utilized by the host plant as well as by the plants later on growing on the same habitat.

In the nineteenth century the above possibility was utilized for the supply of nitrogen in order to
30 achieve a novel increase of the crop yield.

The later further increases in the yield have, however, especially been obtained by means of natural fertilizers and nitrogen-containing synthetic fertilizers. The resulting pollution of the environment makes it desirable to provide alternative possibilities of ensuring the supply of nitrogen necessary for the best possible yields obtainable.

It would thus be valuable to make an improvement possible of the existing nitrogen fixation systems in leguminous plants as well as to allow an incorporation of nitrogen fixation systems in other plants.

The recombinant DNA technique and the plant transformation systems developed render it now possible to provide plants with new qualities in a well-controlled manner. These characteristics can derive from not only the same plant species, but also from all other prokaryotic or eukaryotic organisms. The DNA techniques allow further a quick and specific identification of progeny lines carrying the desired qualities. In this manner a specific plant line can be provided with one or more desired qualities in a quick and well-defined manner.

Correspondingly, plant cells can be provided with well defined qualities and subsequently be maintained as plant cell lines by means of known tissue culture methods. Such plant cells can be utilized for the production of chemical and biological products of particular interest such as dyes, flavours, aroma components, plant hormones, pharmaceutical

products, primary and secondary metabolites as well as polypeptides (enzymes).

A range of factors and functions necessary for biological production of a predetermined gene product are known. Both the initiation and regulation of transcription as well as the initiation and regulation of posttranscriptional processes can be characterised.

At the gene level it is known that these functions are mainly carried out by 5' flanking regions. A wide range of 5' flanking regions from prokaryotic and eukaryotic genes has been sequenced, and in view inter alia thereof a comprehensive knowledge has been provided of the regulation of gene expression and of the sub-regions and sequences being of importance for the regulation of expression of the gene. Great differences exist in the regulatory mechanism of prokaryotic and eukaryotic organisms, but many common features apply to the two groups.

The regulation of the expression of gene may take place on the transsscriptional level and is then preferably exerted by regulating the initiation frequency of transsscription. The latter is well-known and described inter alia by Benjamin Lewin, Gene Expression, John Wiley & Sons, vol. I, 1974, vol. II, Second Edition 1980, vol. III, 1977. As an alternative the regulation may be exerted at the posttranscriptional level, e.g. by the regulation of the frequency of the translation initiation, at the rate of the translation, and of the termination of the translation.

The present invention is based on the surprising finding that 5' flanking regions of root nodule-specific genes, exemplified by the 5' flanking region of the soybean leghemoglobin Lbc_3 gene, can be used for inducible expression of a foreign gene in an alien leguminous plant. The induction and regulation of the promoter is preferably carried out in the form of a regulation and induction at the transcriptional level and differs thereby from the inducability stated in Patent Application No. 86114704.9, the latter inducability preferably being carried out at the translation level.

The transcription of both the Lbc_3 gene of the soybean and of a chimeric Lbc_3 gene transferred to bird's-foot trefoil starts at a low level immediately upon the appearance of the root nodules on the plant roots. Subsequently, a high increase of the transcription takes place immediately before the root nodules turn red. The transcription of a range of other root nodule-specific genes is initiated exactly at this time. The simultaneous induction of the transcription of the Lb genes and other root nodule-specific genes means that a common DNA sequence(s) must be present for the various genes controlling this pattern of expression. Thus the leghemoglobin- c_3 gene is a representative of one class of genes and the promoter and the leader sequence, target areas for activation as well as the control elements of the organ specificity of the Lbc_3 gene are representatives of the control elements of a complete gene class.

The promoter of the 5' flanking regions of the Lb genes functions in soybeans and is responsible for the transcription of the Lb genes in root nodules. It is furthermore known, that the efficiency of both the transcription initiation and the subsequent translation initiation on the leader sequence of the Lb genes is high as the Lb proteins constitute approximately 20% of the total protein content in root nodules.

10 The sequence of 5' flanking regions of the four soybean leghemoglobin genes Lba, Lbc₁, Lbc₂, and Lbc₃ appears from the enclosed sequence scheme, scheme 1, wherein the sequences are stated in such a manner that the homology between the four 5' flanking regions appears clearly.

In the sequence scheme "--" indicates that no base is present in the position in question. The names of the genes and the base position counted upstream from the ATG start codon are indicated to the right 20 of the sequence scheme. Furthermore the important sequences have been underlined.

As it appears from the sequence scheme a distinct degree of homology exists between the four 5' flanking regions, and in the position 23-24 bp upstream 25 from the CAP addition site they all contain a TATATAAA sequence corresponding to the "TATA" box which in eukaryotic cells usually are located a corresponding number of bp upstream from the CAP addition site. Furthermore a CCAAG sequence is 30 present 64-72 bp upstream from the CAP addition site, said sequenc corresponding to the "CCAAT"

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box usually located 70-90 bp upstream from the CAP addition site. From the CAP addition site to the translation start codon, ATG, leader sequences of 52-59 bp are present and show a distinct degree of 5 homology of approx. 75-80%.

In accordance with the present invention it has furthermore been proved, exemplified by the Lbc₃ gene, that the 5' flanking regions of the soybean leghemoglobin genes are functionally active in 10 other plant species. The latter has been proved by fusing the E. coli chloroamphenicol acetyl transferase (CAT) gene with the 5' and 3' flanking regions of the soybean Lbc₃ gene in such a manner that the expression of the CAT gene is controlled 15 by the Lb promoter. This fusion fragment was cloned into the integration vectors pAR1 and pAR22, whereby the plasmids pAR29 and pAR30 were produced. Through homologous recombination the latter plasmids were integrated into the Agrobacterium rhizogenes 20 T DNA region. The transformation of Lotus corniculatus (bird's-foot trefoil) plants, i.e. transfer of the T DNA region, was obtained by wound infection on the hypocotyl. Roots developed from the transformed plant cells were cultivated in vitro and 25 freed from A. rhizogenes bacteria by means of antibiotics. Completely regenerated plants were produced by these root cultures in a conventional manner through somatic embryogenesis or organogenesis.

Regenerated plants were subsequently inoculated 30 with Rhizobium loti bacteria and root nodules for analysis were harvested. Transcription and translation of the chimeric Lbc₃ CAT gene could subse-

quently be detected in root nodules on transformed plants as the activity of the produced chloroamphenicol acetyl transferase enzyme.

The conclusion can subsequently be made that the 5 promoter-containing 5' flanking regions of root nodule-specific genes exemplified by the soybean Lbc₃ promoter are functionally active in foreign plants. The latter is a surprising observation as root nodules are only developed as a consequence 10 of a very specific interaction between the leguminous plant and its corresponding Rhizobium micro-symbiont.

Soybeans produce nodules only upon infection by the species Rhizobium japonicum and Lotus corniculatus 15 only upon infection by the species Rhizobium loti. Soybean and Lotus corniculatus belong therefore to two different cross-inoculation groups, each group producing root nodules by means of two different Rhizobium species. The expression of a chimeric 20 soybean gene in Lotus corniculatus proves therefore an unexpected universal regulatory system applying to the expression of root nodule-specific genes. The regulatory DNA sequences involved can be placed on the 5' and 3' flanking regions of the genes, 25 here exemplified by the 2.0 Kb 5' and 0.9 Kb 3' flanking regions of the Lbc₃ gene. This surprising observation allows the use of root nodule-specific promoters and regulatory sequences in any other plant species and any other plant cell line.

30 In other experiments the 5' flanking region of the nodule-specific N23 gene was fused to the CAT g ne

and the Lbc_3 3' flanking region in such a manner that the expression of the CAT gene is controlled by the N23 promoter. This fusion fragment was cloned into the integration vector pAR22 producing the plasmid N23-CAT which was subsequently recombined into A.rhizogenes and transferred to Lotus corniculatus and Trifolium repens (white clover) by the previously described method. The root nodule-specific expression of the transferred N23-CAT gene obtained in L. corniculatus infected with Rhizobium loti and in T. repens infected with Rhizobium trifolii further demonstrated that expression of root nodule-specific genes is independent of the plant species and Rhizobium species. A universal regulatory system therefore regulates the expression of root nodule-specific genes in the different symbiotic systems formed between legumes and the Rhizobium species of the various cross-inoculation groups.

It is known from European Patent Application EP 122,791.A1 that plant genes from one species, by Agrobacterium mediated transformation, can be transferred into a different plant species. It is also known from EP 122,791.A1 that a transferred gene encoding the seed storage protein "Phaseolin" can be expressed into tobacco and alfalfa. From the literature it is also known that this expression is seed specific (Sengupta-Gopalan et al. 1985, Proc. Natl. Acad. Sci. 82, 33203324).

The present invention therefore relates to a novel method for the expression of transferred genes in a root nodule-specific manner, using DNA regulatory

sequences from the 5' promoter region, the coding region, or the 3' flanking region of root nodule-specific genes, here exemplified by the leghemoglobin Lbc₃ gene and the N23 gene. This method is 5 distinct from both the method of Agrobacterium mediated transformation and expression of the seed storage protein phaseolin gene characterised in EP 122,791.A1. Expression of the transferred phaseolin gene in EP 122,791.A1 only demonstrates that the 10 phaseolin gene family with its particular regulatory requirements can be expressed in tobacco and alfalfa. It does not demonstrate nor predict that any other genes with their particular regulatory requirements can be expressed in any other plants or 15 plant tissue.

An object of the present invention is to provide a possibility of expressing desired genes in plants, parts of plants, and plant cell cultures.

A further object of the invention is to render it 20 possible to express genes of any origin by the control of an inducible root nodule-specific promoter.

A particular object of the invention is to provide a possibility of expressing desired genes in leguminous plants. 25

A still further particular object of the invention is to provide a possibility of expressing root nodule-specific genes in non-leguminous plants.

Further objects of the invention are to improve the

existing nitrogen-fixing systems in leguminous plants as well as to incorporate nitrogen-fixing systems in other plants.

A further object of the invention is to provide a 5 possibility of in certain cases allowing the use of specific sequences of the 3' flanking region, of the coding sequence, and of intervening sequences to influence the regulation of the root nodule-specific promoter.

10 Furthermore it is an object of the invention to provide plasmids comprising the above mentioned inducible plant promoter.

Further objects of the invention appear immediately from the following description.

15 The method according to the invention for the expression of genes in plants, parts of plants, and plant cell cultures is carried out by introducing into a cell thereof a recombinant DNA segment containing both the gene to be expressed and a 5' 20 flanking region comprising a promoter sequence, and optionally a 3' flanking region, and culturing of the transformed cells in a growth medium, said method being characterised by using as the recombinant DNA segment a DNA fragment comprising an 25 inducible plant promoter (as defined) from root nodule-specific genes. If desired the transformed cells are regenerated to plants.

The method according to the invention allows in a well defined manner an expression of foreign genes

in plants, parts of plants, and plant cell cultures, in this connection especially genes providing the plants with desired properties such as for instance a resistance to plant diseases and increased content 5 of valuable polypeptides.

A further use is the preparation of valuable products such as for instance dyes, flavourings, plant hormones, pharmaceutical products, primary and secondary metabolites, and polypeptides by means 10 of the method according to the invention in plant cell cultures and plants.

By using the method according to the invention for the expression of root nodule-specific genes it is possible to express root nodule-specific genes 15 necessary for the formation of an active nitrogen-fixing system both in leguminous plants and other plants. The correct developmental control, cf. Example 8, allows the establishment of a symbiotic nitrogen-fixing system in non-leguminous plants. In 20 this manner it is surprisingly possible to improve the existing nitrogen-fixing systems in leguminous plants as well as to incorporate nitrogen-fixing systems in other plants.

The use of the method according to the invention 25 for the expression of foreign genes in root nodules renders it possible to provide leguminous plants with improved properties such as resistance to herbicides and resistance to diseases and pest.

According to a particular embodiment of the method 30 according to the invention a DNA fragment is used

which comprises an inducible plant promoter and which is identical with, derived from, or comprises 5' flanking regions of leghemoglobin genes. In this manner the expression of any gene is obtained.

5 Examples of such DNA fragments are DNA fragments of the four 5' flanking regions of the soybean leghemoglobin genes, viz.

Lba with the sequence:

10 GAGATACATT ATAATAATCT CTCTAGTGTC TATTTATTAT TTTATCTGGT
 GATATATACC TTCTCGTATA CTGTTATTTT TCAATCTTG TAGATTTACT
 TCTTTTATT TTATAAAAAAA GACTTTATTT TTTAAAAAAA AATAAAGTGA
 ATTTTGAAAA CATGCTCTTT GACAATTTC TGTTCCCTT TTCATCATTG
 GGTAAATCT CATACTGCCT CTATTCAATA ATTGGGCTC AATTAAATTA
 GTAGAGTCTA CATAAAATT ACCTTAATAG TAGAGAATAG AGAGTCTTGG
 AAAGTTGGTT TTTCTCGAGG AAGAAAGGAA ATGTTAAAAA CTGTGATATT
 15 TTTTTTTGCG ATTAATAGTT ATGTTTATAT GAAAACGTGAA AATAAATAAA
 CTAACCATAT TAAATTTAGA ACAACACTTC AATTATTTT TTAATTGAT
 TAATTAAAAA ATTATTTGAT TAAATTTTT AAAAGATCGT TGTTCTTCT
 TCATCATGCT GATTGACACC CTCCACAAGC CAAGAGAAAC ACATRAGCTT
 TGTTTTCTC ACTCTCCAAG CCCTCTATAT AAACAAATAT TGGAGTGAAG
 TTGTTGCATA ACTTGCATCG AACAAATTAAT AGAAATAACA GAAAATTAAA
 AAAGAAATAT G,

20 Lbc₁ with the sequence:

TTCTCTTAAT ACAATGGAGT TTTTGTGAA CATAACATACA TTTAAAAAAA
 AATCTCTAGT GTCTATTTAC CCGGTGAGAA GCCTTCTCGT GTTTACACA
 CTTTAATATT ATTATATCCT CAACCCCCACA AAAAGAATA CTGTTATATC
 TTTCCAAACC TGTAGATTAA TTTATTATT TATTATTTT TACAAAGGAG
 ACTTCAGAAA AGTAATTACA TAAAGATAGT GAACATCATT TTATTATTA
 TAATAAACCT TAAAATCAA CTTTTTATA TTTTTGTAA CCCTTTCAT
 25 TATTGGGTGA AATCTCATAG TGAAGCCATT AAATAATTG GGCTCAAGTT
 TTATTAGTAA AGTCTGCATG AAATTAACT TAACAATAGA GAGAGTTTC
 GAAAGGGAGC GAATGTTAAA AAGTGTGATA TTATATTTA TTTCGATTAA
 TAATTATGTT TACATGAAAA CATACAAAAA AATACTTTA AATTCAAGAT
 AATACTTAAA ATATTTATT GCTTAATTGA TTAACTGAAA ATTATTTGAT
 TAGGATTTG AAAAGATCAT TGGCTCTTCG TCATGCCGAT TGACACCCCTC
 CACAAGCCAA GAGAAACTTA AGTTGTAAC TTTCTCACTC CAAGCCTTCT
 ATATAAACAT GTATTGGATG TGAAGTTATT GCATAACTTG CATTGAACAA
 30 TAGAAATATAA CAAAAAAAG TAAAAAGTA GAAAAGAAAT ATG,

Lbc₂ with the sequence:

TCGAGTTTT ACTGAACATA CATTATTAA AAAAAACTCT CTAGTGTCCA
 TTTATTCGGC GAGAACCTT CTCGTCTT ACACACTTA ATATTATTAT
 ATCCCCACCC CCACCAAAAA AAAAAAAACT GTTATATCTT TCCAGTACAT
 TTATTTCTTA TTTTACAAA GGAAACTTCA CGAAAGTAAT TACAAAAAAG
 5 ATAGTGAACA TCATTTTT AGTTAAGATG AATTTAAAAA TCACACTTT
 TTATTTTT TTGTTACCTT TTTCATTATT GGGTGAAATC TCATAGTGA
 ACTATTAAT AGTTTGGCT CAAGTTTAT TAGTAAAGTC TGCATGAAAT
 TTAACTTAAT AATAGAGAGA GTTTGGAAA GGTAACGAAT GTTAGAAAGT
 GTGATATTAT TATAGTTTA TTTAGATTAA TAATTATGTT TACATGAAA
 TTGACAATTT ATTTTAAAAA TTCAGAGTAA TACTAAATT ACTTATTTAC
 TTTAAGATTT TGAAAAGATC ATTGGCTCT TCATCATGCC GATTGACACC
 10 CTCCACAAGC CAAGAGAAAC TTAAGTTGA ATTTTCTAA CTCCAAGCCT
 TCTATATAAA CACGTATTGG ATGTGAAGTT GTTGACATAAC TTGCATTGAA
 15 CAATAGAAAT AACAAACAAAG AAAATAAGTG AAAAAAGAAA TATG,

and Lbc₃ with the sequence:

TATGAAGATT AAAAAATACA CTCATATATA TGCCATAAGA ACCAACAAAA
 GTACTATTTA AGAAAAGAAA AAAAAAACCT GCTACATAAT TTCCATCTT
 15 GTAGATTTAT TTCTTTATT TTTATAAAGG AGAGTTAAAA AAATTACAAA
 ATAAAAATAG TGAACATCGT CTAAGCATT TTATATAAGA TGAATTTTAA
 AAATATAATT TTTTGCTCA AATCGTATGT ATCTTGTCTT AGAGCCATT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTTCCT CCGAGTTGA
 TATAAAAAAA ATTGTTCCC TTTGATTAT TGGATAAAAT CTCGTAGTGA
 CATTATATTA AAAAAATTAG GGCTCAATT TTATTAGTAT AGTTTGCATA
 20 AATTTTAACT TAAAAATAGA GAAAATCTGG AAAAGGGACT GTTAAAAGT
 GTGATATTAG AAATTGTCG GATATATTAA TATTTTATT TATATGGAAA
 CTAAAAAAAT ATATATTAAA ATTTTAAATT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTTGAAAAGA TGATTGTCTC
 TTCACCATAC CAATTGATCA CCCTCCTCCA ACAAGCCAAG AGAGACATAA
 GTTTTATTAG TTATTCTGAT CACTCTCAA GCCTCTATA TAAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACTTGCAT TGAACAAATTA ATAGAAATAA
 25 CAGAAAAGTA GAAAAGAAAT ATG.

A further embodiment of the method according to the invention uses a DNA fragment identical with, derived from or comprising 5' flanking regions of the Lbc₃-5'-3'-CAT gene with the sequence:

30 TATGAAGATT AAAAAATACA CTCATATATA TGCCATAAGA ACCAACAAAA
 GTACTATTTA AGAAAAGAAA AAAAAAACCT GCTACATAAT TTCCATCTT
 GTAGATTTAT TTCTTTATT TTTATAAAGG AGAGTTAAAA AAATTACAAA
 ATAAAAATAG TGAACATCGT CTAAGCATT TTATATAAGA TGAATTTTAA
 AAATATAATT TTTTGCTCA AATCGTATGT ATCTTGTCTT AGAGCCATT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTTCCT CCGAGTTGA

TATAAAAAAA ATTGTTTCCC TTTGATTAT TGGATAAAAT CTCGTAGTGA
 CATTATATTA AAAAATTAG GGCTCAATT TTATTAAGTAT AGTTTGCAATA
 AATTTTAAC TAAAATAGA GAAAATCTGG AAAAGGGACT GTTAAAAAGT
 GTGATATTAG AAATTTGTGCG GATATATTA TATTTTATT TATAATGGAAA
 CTAAAAAAAT ATATATTAATTA ATTTTAATT CAGAATAATA CTAAATTTAT
 5 TTATTTACTG AAAATGAGTT GATTTAAGTT TTTGAAAAGA TGATTGTCTC
 TTCRCCCATAC CAATTGATCA CCCTCCCTCCA ACCTAGCCCTAG AGAGACATAA
 GTTTTATTAG TTATTTGTGAT CACTCTTCAA GCCTTCTATA TAATTAAGTA
 TTGGATGTGA AGTTGTTGCA TRACTTGCAT TGAACTTAA ATAAGAAATAA
 CAGAAAAAGTA GAATTCTAAA ATG

10 A still further preferred embodiment of the method according to the invention uses a DNA fragment identical with, derived from or comprising 5' flanking regions of the N23 gene with the sequence

10 20 30 40 50 60 70
GAATTCGAGCTGCCCGGGATCGATCCTCTAGACTGGACCTGCAGCCCCAAGCTGGATCAATCAATTAA
Sall
 15 80 90 100 110 120 130 140
TTCTATTGAGACACCGATTGACAATTTTACATTATGAGACTATTTGGTTTTTATTGATCCAAA
 150 160 170 180 190 200 210
AAATTTAACGCTTAGATGATGATGAAATTGAAANNAATTGTATAATTNTGAAAAGTTNNNNNGTTA
 220 230 240 250 260 270 280
ATGAATGCTATGATATTGATGGCTTGTATNNCAAGATTGAAAGTATTAAAGAGAACTGTTAAGAAA
 290 300 310 320 330 340 350
AGAAGTTAGCACACCAATAGAAGTATTGAGTTATTTAAACTTTAGATTCTTTCAAATGTTACATTG
 360 370 380 390 400 410 420
CATATAGAATTATTATTGACAATCCTTATAACAGTTGCTACTGTTGAAAGACGTTCTCAAATTAAATT
 430 440 450 460 470 480 490
 20 ACTTAAATCATATCTAAATCAACATGTTACAAGATAGATTGAATGAGTTAGTTATTTATCTATTGAA
 500 510 520 530 540 550 560
AGTAAAGTGTAGAATTGTTGATTATAAAACTCTGATAATGATTTGCAAGTTAAAAAAACTAGAAGAT
 570 580 590 600 610 620 630
TAATATAAAAATTGATATTTTATATAATATAAGTCTCTTAAATTCTGTAAAAAAAGACATTTT
 640 650 660 670 680 690 700
AAATAATAAAATAAGCAACTCTTAATTAAATGAAACATCCCTTGTAAACCGAATCTCCATAATGT
 710 720 730 740 750 760 770
AAAAATTAATGCTTGATGGAAGTTTAAATTGTTGTTACTCAACTCAAAAGGGTTGTAATTTTTT
 780 790 800 810 820 830 840
 25 TATCATTATATGTTGAAATATGAAATGCACTAGTAATTGTTAATGATAAAATATAATTCTACAGATAT

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23

850 860 870 880 890 900 910
ATTTCTGCTTGGCAACTCGTGAGAATTGAATATAATTATAAGATGAAAGTCGTTACAATTTTTTT

920 930 940 950 960 970 980
AGAATAAAATTTATACAACTCCTAGATTTGTTATAAAATTCAACATATTGTATGAGTATAAACAT

990 1000 1010 1020 1030 1040 1050
GAGCACACACCAAACTAGTCTCAAATTAGTAAGGTGCTAATTATTAGCGGCTAGCTAAGTAACCAAGTA
Del

ATTAATG

5 In a particularly preferred embodiment of the method according to the invention a 3' flanking region of root nodule-specific genes is furthermore used, in particular sequences of the 3' flanking region capable of influencing the activity or regulation of
10 a promotor of the root nodule-specific genes or the transcription termination, or capable of influencing the yield of the desired gene product in another manner.

Examples of such 3' flanking regions are the four
15 3' flanking regions of the soybean leghemoglobin
genes, viz.

Lba with the sequence:

1590 1620
 TAA TTA GTA TCT ATT GCA GTA AAG TGT AAT AAA TAA ATC TTG
 1650 1680
 TTT CAC TAT AAA ACT TGT TAC TAT TAG ACA AGG GCC TGA TAC AAA ATG TTG GTT AAA ATA
 1710 1740
 20 ATG GAA TTA TAT AGT ATT GGA TAA AAA TCT TAA GGT TAA TAT TCT ATA TTT GCG TAG GTT
 1770 1800
 TAT GCT TGT GAA TCA TTA TCG GTA TTT TTC CTT TCT GAT AAT TAA TCG GTA AAT TA
 1830 1860
 ACA AAT AAG TTC AAA ATG ATT TAT ATG TTT CAA AAT TAT TTT AAC AGC AGG TAA AAT GTT
 ATT TGG TAC GAA AGC TAA TTC GTC GA

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24

Lbc₁ with the sequence:

1320
TAA/TG AGG ATC TAC TGC ATT GCC GTA

1350 1380
AAG TGT AAT AAA TAA ATC TTG TTT CAA CTA AAA CTT GTT ATT AAA CAA GTT CCC TAT ATA

1410 1440
AAT GTT GGT TAA AAT AAG TAA ATT TCA TTG TAT TGG ATA AAC ACT TTT AAG TTA TAT ATT

1470 1500
5 TCC ATA TAT TTA CGT TTG TGA ATC ATA ATC GAT ACT TTA TAA AAA TAA ATT CCA AAT AAT
TTA TAC GTT TTA AAA ATT ATT TT

Lbc₂ with the sequence:

TAG/GAT CTA CTA TTG CCG TCA AGT
1140

GTA ATA AAT AAA TTT TGT TTC ACT AAA ACT TGT TAT TAA ACA AGT CCC CGA TAT ATA AAT
1170 1200
10 GTT GGT TAA AAT AAG TAA ATT ATA CGG TAT TGA TAA ACA ATC TTA AGT TTT ATA TAT AGT
1230 1260

TCC ATA TAC TAA AGT TTG TGA ATC ATA ATC GA
1290

and Lbc₃ with the sequence:

TAG/GAT CTA CAA TTG CCT TAA AGT GTA ATA AAT AAA
990 1020

TAT TAT TTC ACT AAA ACT TGT TAT TAA ACC AAG TTC TCG ATA TAA ATG TTG GTT AAA CTA
1050 1080

15 AGT AAA TTA TAT GGT ATT GGA TAA ACA ATC TTA AGC TT
1110

This sequence is positioned on the 0.9 Kb 3' flanking region used according to the invention. A particular embodiment of the invention is therefore

the use of sequences of this region exerting or mediating the regulation characterised by the invention of root nodule-specific promoter regions.

In a preferred embodiment of the method according to the invention a region is used of the coding sequence or intervening sequence of root nodule-specific genes, in particular sequences of the coding sequence or the intervening sequence capable of influencing the regulation of a promotor of the root nodule-specific genes or capable of influencing the yield of the desired gene product in another manner.

Examples of such coding sequences and intervening sequences are the four leghemoglobin genes of soybean, viz.

Lba with the sequence:

120
VAL
ATG/GTT

ALA PHE THR GLU LYS GLN ASP ALA LEU VAL SER SER SER PHE GLU ALA PHE LYS ALA ASN GCT TTC ACT GAG AAG CAA GAT GCT TTG GTG AGT AGC TCA TTC GAA GCA TTC AAG GCA AAC	150	180
ILE PRO GLN TYR SER VAL VAL PHE TYR THR SER ATT CCT CAA TAC AGC GTT GTG TTC TAC ACT TC/G TAA GTT TTC TCT CTA AGC ATG TGT CTT	210	240
CCA TTC TAT GTT TTT CTT TTG GAA ATT TGT TGT GTT TGA AAA AAG ATA TAT TGT TAA TGT 20	270	300
GAG TGG TTT TGG TTT GAT TAA AAA TGA ATAG/G ATA CTG GAG AAA GCA CCT GCA GCA AAG GAC 330	330	360
LEU PHE SER PHE ILE ALA ASN GLY VAL ASP PRO THR ASN PRO LYS LEU THR GLY HIS ALA TTG TTC TCA TTT CTA GCA AAT GGA GTA GAC CCC ACT AAT CCT AAC CTC ACG GCC CAT GCT 390	390	420
GLU LYS LEU PHE ALA LEU GAA AAG CTT TTT GCA TTG/GTAA GTA TCA CCC AAC TAA AAT TAT AAC TAT TTT ATG TGA 450	450	480
TTA ATT TTA AGA TTA AGC ATC ATG TAT TTT AAC ACT CTT AAA ACA TCA ATG AAC ATT AAT 510	510	540
TGT TTG AAT TGT ATT TTA TAT TTT TGC CAT ATC TTG AAC TAG GAA TAG TAT ATA AAT TTC 570	570	600
TAT TAG TAT TTG TTG ATA ATT ATT TTT CTT TCA TAA CTA TCT TGT CAC ATA TTA TAT ATT 63	63	660

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690

VAL ARG ASP SER ALA GLY GLN LEU LYS ALA SER GLY THR VAL VAL ALA
TTT TGA ATT GTAG/GTG CGT GAC TCA GCT GGT CAA CTT AAA GCA AGT GGA ACA GTG GTG GCT

750

ASP ALA ALA LEU GLY SER VAL HIS ALA GLN LYS ALA VAL THR ASP PRO GLN PHE VAL
GAT GCC GCA CTT GGT TCT GTT CAT GCC CAA AAA GCA GTC ACT GAT CCT CAG TTC GTG/GT

810

ATG ATA AAT AAT GAA ATG TTA TAA TAA ATT ATG CAT ACT TCA ATT TTT CAT GGA GCA GTA

870

TAA TGA TCA ACA CAC ACT TCT TTT GTT TCA TGC ATT TGA TAA CTA CAA TCT TAA AAT GTT

930

5 GCA ATC TTA AAA ATA GTA TTA AAA ATA TAA CAT TTA ATT AGC TCA TCA ATA TTT TTC TGT

990

TGC AAT TTT TTA TGA AAA AAT TAT AAT TAT GAA TTC TTT GAG CAA TGT TTA ATT AAA AAA

1050

TTG ATT TAA TAA TGA AAT AAC TAA GCT ACC TCT GTC TCG TTT TTC ATT TAA ACT ATG ACA

1110

TAA ACA ATG AAT AAA GTA AAC TAA ACC ACC ATG ACA TGT TTA TTT TTG AAT GAG GTT ATT AAT

1170

AAT TTT TTT TCA CTA TCT ATT GCA ATG TTC ATT GAT TAT CAA TTA TCT TGG TTG CAT TGA

1230

10 TTC TCT CGA TTT TTT TCT TGA GGT TAA GCT TCA GTT CAA TAT ATA TTC ATT TTT TGA TAA

1290

AAA AAA ATA GTA CAA TAT ATT TTC ATT TAG CTG ATC ATA TTT ATT TAA GTT CAA CTT AAA

1350

ATT TTA TAG ATG TTA ATT GAT ATA ATT TGT TGA GAT GAT GAG ARG ACC AAT ACC ATT ACG

1410

TAC TCT TTT GAA AGT GTT ATA TGG ATT TTA ATT ATA AGG AAA AAT GTA AGA GCT AAA CCA

1470

15 VAL VAL LYS GLU ALA LEU LEU LYS THR ILE LYS ALA ALA VAL
TTG CTG ATG ATT TTG AAG/GTG GTT AAA GAA GCA CTG CTG AAA ACA ATA AAG GCA GCA GTT

1530

GLY ASP LYS TRP SER ASP GLU LEU SER ARG ALA TRP GLU VAL ALA TYR ASP GLU LEU ALA
GGG GAC AAA TGG AGT GAC GAG TTG AGC CGT GCT TGG GAA GTA GCC TAC GAT GAA TTG GCA

1590

ALA ALA ILE LYS LYS ALA
GCA GCT ATT AAG AAG GCA TAA

The amino acid sequence of the Lba protein is indicated above the coding sequence,

Lbc₁ with the sequence:

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27

180
GLY
ATG/GCT

The amino acid sequence of the Lbc1 protein is indicated above the coding sequence,

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28

Lbc₂ with the sequence:

GLY
G/GGT
180

ALA PHE THR GLU LYS GLN GLU ALA LEU VAL SER SER SER PHE GLU ALA PHE LYS ALA ASN
GCT TTC ACT GAG AAG CAA GAG GCT TTG GTG AGT AGC TCA TTC GAA GCA TTC AAG GCA AAC
210 240

ILE PRO GLN TYR SER VAL VAL PHE TYR THR SER
ATT CCT CAA TAC AGC GTT GTG TTC TAC ACT TC/GTA AGT TTT CTC TTA AAG CAT GTA TCT
270 300

5 TTC ATT CTC TGT TTT TCC TTT CGA CAT TTT TTG TGT TTG AAA AGA GAT AGT GTC AAT GTG
330 360

ILE LEU GLU LYS ALA PRO ALA ALA LYS
AGT GGG TAT TTT TTT TTA TTA AAA ATT AAC AG/G ATA CTG GAG AAA GCA CCC GCA GCA AAG
390 420

ASP LEU PHE SER PHE LEU SER ASN GLY VAL ASP PRO SER ASN PRO LYS LEU THR GLY HIS
GAC TTG TTC TCG TTT CTA TCT ATT GGA GTA GAT CCT AGT ATT CCT AAG CTC ACG GGC CAT
450 480

ALA GLU LYS LEU PHE GLY LEU
GCT GAA AAG CTT TTT GGA TTG/GTA AGT ATC ATC CAA CTA AAA TTA TAG CTA TTT TAT GTG
510 540

10 ATT ATT TTT AAG ATT AAA CAT GTA TTT AAC ACT CTT AAA CAT GTA TTT AAC ACT CTT AAG
570 600

ATT AAA CAT GTA TTT AAC TAA AAC ATG TAT TTG CTG ATT ATT TTT TTT TTA TAA TTA TCT
630 660

VAL ARG ASP SER ALA GLY GLN LEU LYS ALA
TGT CAC ATA TTA TAT ATT TTT TGA ATT GTA G/GTG CGT GAC TCA GCT GGT CAA ATT AAA GCA
690 720

ASN GLY THR VAL VAL ALA ASP ALA ALA LEU GLY SER ILE HIS ALA GLN LYS ALA ILE THR
AAT GGA ACA GTA GTG GCT GAT GCC GCA CTT GGT TCT ATC CAT GCC CAA AAA GCA ATC ACT
750 780

15 ASP PRO GLN PHE VAL
GAT CCT CAG TTC GTG/GT ATG ATA ATT ATT AAA ATG TTA CAA TAA ATG CAC ATA TAC TTA
810 840

AAT TTT ACA TGG TGC AGT GTT ATG ATC ATT TTT GTT TAG TAA TGA ATT TAC TTA AAA
870 900

TCT TAA ATT ATG TAC TTT TTG AAA GTT TTA TAT GGA ATT TTA ATT ATA CGG AAA AAT GTA
930 960

VAL VAL LYS GLU ALA LEU LEU LYS THR
AGA CCT ATT CCA TTA GTG ATG TTT TGT CTG TAG/GTG GTT AAA GAA GCA CTG CTG AAA ACA
990 1020

LE LYS GLU ALA VAL GLY ASP LYS TRP SER ASP GLU LEU SER SER ALA TRP GLU VAL ALA
ATA AAG GAG GCA GTT GGG GAC AAA TGG AGT GAT GAA TTG AGC AGT GCT TGG GAA GTA GCC
1050 1080

20 TYR ASP GLU LEU ALA ALA ILE LYS LYS ALA PHE
TAT GAT GAA TTG GCA GCA GCT ATT AAG AAG GCA TTT TAC
1110

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29

The amino acid sequence of the Lbc₂ protein is indicated above the coding sequence,

and Lbc₃ with the sequence:

GLY ALA PHE THR ASP
G/GGT GCT TTC ACT GAT
120

LYS GLN GLU ALA LEU VAL SER SER SER PHE GLU ALA PHE LYS THR ASN ILE PRO GLN TYR
5 AAG CAA GAG GCT TTG GTG AGT AGC TCA TTT GAA GCA TTC AAG ACA AAC ATT CCT CAA TAC
150 180

SER VAL VAL PHE TYR THR SER
ACT GTT GTG TTC TAC ACC TC/GTA AGT ATT CTA TCT AAA TTA TGT GTC TTA TTG TAT GTT
210 240

TAA CTT TCG TGG TTT GTG TTT GAA AAA AAG ATA TAT ATT GTT AAT GTG AGT GGT TTT
270 300

ILE LEU GLU LYS ALA PRO VAL ALA LYS ASP LEU PHE SER
GGT TTG ACT AAA AAT GAA TAG/G ATA CTG GAG AAA GCA CCT GTC GCA AAG GAC TTG TTC TCA
330 360

10 PHE LEU ALA ASN GLY VAL ASP PRO THR ASN PRO LYS LEU THR GLY HIS ALA GLU LYS LEU
TTT CTA GCT AAT GGA GTA GAC CCC ACT AAT CCT AAG CTC ACG GCC CAT GCT GAA AAA CTT
390 420

PHE GLY LEU
TTT GGA TTG/GT AAG TAT CCA GCC TAC TAA AAT TAA AAT CCT ATT AGT ATT TTT TAT TAT
450 480

VAL ARG ASP SER
TTT TCT TCC ATG ATT GTC TTG TCA CAT ATT ATA TAT TTT TTG AAT TAT AG/GTA CGT GAT TCA
510 540

ALA GLY GLN LEU LYS ALA SER GLY THR VAL VAL ILE ASP ALA ALA LEU GLY SER ILE HIS
GCT GGT CAA CTT AAA GCA AGT GGA ACA GTG GTG ATT GAT GCC GCA CTT GGT TCT ATC CAT
570 600

15 ALA GLN LYS ALA ILE THR ASP PRO GLN PHE VAL
GCC CAA AAA GCA ATC ACT GAT CCT CAA TTT GTG/G TAT GAT AAA TAA TGA AAA GCT ACA
630 660

ATA AAT GCA CAA ATA CTT AAT TTT ACA TAG TGC ACT GCT ATA TGA TCA TCA CTT TTG CTT
690 720

AGT AAT GAA TTT ACT TTT TTT TAC AGA AGT AAT GGA TTT ACT TAA AAT CTT AAA TTA
750 780

TGT ACT TCT TTA AAG AGT TTT GTC TGG AAT TTT AAT TAT AGG AAA AAT GTC AGA GCT AAA
810 840

VAL VAL LYS GLU ALA LEU LEU LYS THR ILE LYS GLU ALA
CCA TTG CTG ATG ATT TCG AAG/GTG GTT AAA GAA GCA CTG CTG AAA ACA ATA AAG GAG GCA
870 900

20 VAL GLY ASP LYS TRP SER ASP GLU LEU SER SER ALA TRP GLU VAL ALA TYR ASP GLU LEU
GTT GGG GAC AAA TGG AGT GAC GAG TTG AGC AGT GCT TGG GAA GTA GCC TAT GAT GAA TTG
930 960

ALA ALA ALA ILE LYS LYS ALA PHE
GCA GCA GCT ATT AAG AAG GCA TTT TAG

The amino acid sequence of the Lbc₃ protein is indicated above the coding sequence.

The present invention furthermore deals with a novel DNA fragment comprising an inducible plant promoter to be used when carrying out the method according to the invention, said DNA fragment being characterised by being identical with, derived from or comprising a 5' flanking region of root nodule-specific genes. Examples of such DNA fragments are DNA fragments being identical with, derived from or comprising a 5' flanking region of plant leghemoglobin genes. Preferred examples are according to the invention DNA fragments being identical with, derived from or comprising a 5' flanking region of the four soybean leghemoglobin genes, viz.:

Lba with the sequence:

GAGATACATT ATAATAATCT CTCTAGTGTCT TATTTATTAT TTTATCTGGT
GATATATACC TTCTCGTATA CTGTTATTT TTCAATCTG TAGATTTACT
20 TCTTTTATTT TTATAAAAAA GACTTTATTT TTTTAAAAAA AATAAAAGTGA
ATTTTGAAAA CATGCTCTT GACAATTTC TGTTTCCCTT TTCATCATGG
GGTTAAATCT CATACTGCCT CTATTCAATA ATTTGGGCTC AATTTAATTA
GTAGAGTCTA CATAAAATTT ACCTTAATAG TAGAGAATAG AGAGTCTTGG
AAAGTTGGTT TTTCTCGAGG AAGAAAGGAA ATGTTAAAAA CTGTGATATT
TTTTTTTGG ATTAAATAGTT ATGTTTATAT GAAAATGAA AATAAAATAAA
25 CTAACCATAT TAAATTTAGA ACAACACTTC ATTATTTTT TTAATTTGAT
TAATTAAAAA ATTATTTGAT TAAATTTTT AAAAGATCGT TGTTCTTCT
TCATCATGCT GATTGACACC CTCCACAAGC CAAGAGAAAC ACATAAGCTT
TGTTTTCTC ACTCTCCAAG CCCTCTATAT AACAAATAT TGGAGTGAAG
TTGTTGCATA ACTTGCATCG AACAAATTAAT AGAAATTAACG GAAATTTAAA
AAAGAAATAT G.

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Lbc₁ with the sequence:

TTCTCTTAAT ACAATGGAGT TTTTGTGAA CATAACATACA TTTAAAAAAA
AATCTCTAGT GTCTATTAC CCGGTGAGAA GCCTTCTCGT GTTTTACACA
CTTTAATATT ATTATATCCT CAACCCCCACA AAAAAGAATA CTGTTATATC
5 TTTCCAAACC TGTAGATTAA TTTATTATT TATTATTTTT TACAAAGGAG
ACTTCAGAAA AGTAATTACA TAAAGATAGT GAACATCATT TTATTATTAA
TAATAAAACTT TAAAATCAA CTTTTTTATA TTTTTGTTA CCCTTTCAT
TATTGGGTGA AATCTCATAG TGAAGCCATT AAATAATTG GGCTCAAGTT
TTATTAGTAA AGTCTGCATG AAATTAACT TAACAATAGA GAGAGTTTC
GAAAGGGAGC GAATGTTAAA AAGTGTGATA TTATATTAA TTTCGATTAA
TAATTATGTT TACATGAAAA CATAACAAAA AATACTTTA AATTCAAGAT
AATACTTAA ATATTATTG GCTTAATTGA TTAACTGAAA ATTATTGAT
10 TAGGATTTCG AAAAGATCAT TGGCTCTTCG TCATGCCGAT TGACACCCCTC
CACAAAGCCAA GAGAAACTTA AGTTGTAAC TTTCTCACTC CAAGCCTCT
ATATAAAACAT GTATTGGATG TGAAGTTATT GCATAACTTG CATTGAACAA
TAGAAAAATAA CAAAAAAAG TAAAAAAGTA GAAAAGAAAT ATG,

Lbc₂ with the sequence:

TCGAGTTTT ACTGAACATA CATTTATTAA AAAAAGCTCT CTAGTGTCCA
TTTATTCGGC GAGAACGCTT CTCGTGCTT ACACACTTTA ATATTATTAT
15 ATCCCCACCC CCACCAAAAA AAAAAGCTCT GTTATATCTT TCCAGTACAT
TTATTCTTA TTTTACAAA GGAAGACTCA CGAAAGTAAT TACAAAAAGA
ATAGTGAACA TCATTTTTT AGTTAAGATG AATTTTAAA TCACACTTT
TTATATTTC TTGTTACCCCT TTTCATTATT GGGTGAATC TCATAGTGA
ACTATTAAT AGTTGGGCT CAAGTTTAT TAGTAAAGTC TGCATGAAAT
TTAACCTTAAT AATAGAGAGA GTTTTGGAAA GGTAACGAAT GTTAAAGT
GTGATATTAT TATAGTTTA TTAGATTAA TAATTATGTT TACATGAAAA
TTGACAAATT ATTTTAAATC TTCAGAGTAA TACTTAAATT ACTTATTAC
20 TTTAAGATT TGAAAAGATC ATTGGCTCT TCATCATGCC GATTGACACC
CTCCACAGC CAAGAGAAAC TTAAGTTGTA ATTTTCTAA CTCCAAGCCT
TCTATATAAA CACGTATTGG ATGTGAAGTT GTGCATAAC TTGCATTGAA
CAATGAAAT AACAAACAAAG AAAATAAGTG AAAAAGAAA TATG,

and Lbc₃ with the sequence:

TATGAAGATT AAAAAATACA CTCATATATA TGCCATAAGA ACCAACAAAA
 GTACTATTTA AGAAAAGAAA AAAAAAACCT GCTACATAAT TTCCAATCTT
 GTAGATTTAT TTCTTTTATT TTTATAAAGG AGAGTTAAAA AAATTACAAA
 ATAAAAAATAG TGAACATCGT CTAAGCATT TTATATAAAGA TGAATTAA
 AAATATAATT TTTTGTCCTA AATCGTATGT ATCTTGTCTT AGAGCCATT
 5 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTCCT CCGAGTTGAA
 TATAAAAAAA ATTGTTCCC TTTGATTAT TGGATAAAAT CTCGTAGTGA
 CATTATATTA AAAAAATTAG GGCTCAATT TTATTAGTAT AGTTGCATA
 AATTTTAACT TAAAGATAGA GAAAATCTGG AAAAGGGACT GTAAAAAAGT
 GTGATATTAG AAATTTGTCG GATATATTAA TATTTTATT TATATGGAAA
 CTAAAAAAAT ATATATTAA ATTAAATTT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTTGAAAAGA TGATTGTCTC
 TTCACCACAC CAATTGATCA CCCTCCTCCA ACAAGCCAG AGAGACATAA
 10 GTTTTATTAG TTATTCTGAT CACTCTCAA GCCTTCTATA TAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACTTGCAT TGAACAAATTA ATAGAAAATAA
 CAGAAAAGTA GAAAAGAAAT ATG.

Another example of a preferred DNA fragment according to the invention is a DNA fragment which is
 15 identical with, derived from or comprises 5' flanking regions of the Lbc₃-5'-3'CAT gene with the sequence

TATGAAGATT AAAAAATACA CTCATATATA TGCCATAAGA ACCAACAAAA
 GTACTATTTA AGAAAAGAAA AAAAAAACCT GCTACATAAT TTCCAATCTT
 GTAGATTTAT TTCTTTTATT TTTATAAAGG AGAGTTAAAA AAATTACAAA
 20 ATAAAAAATAG TGAACATCGT CTAAGCATT TTATATAAAGA TGAATTAA
 AAATATAATT TTTTGTCCTA AATCGTATGT ATCTTGTCTT AGAGCCATT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTCCT CCGAGTTGAA
 TATAAAAAAA ATTGTTCCC TTTGATTAT TGGATAAAAT CTCGTAGTGA
 CATTATATTA AAAAAATTAG GGCTCAATT TTATTAGTAT AGTTGCATA
 AATTTTAACT TAAAGATAGA GAAAATCTGG AAAAGGGACT GTAAAAAAGT
 GTGATATTAG AAATTTGTCG GATATATTAA TATTTTATT TATATGGAAA
 25 CTAAAATAT ATATATTAA ATTAAATT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTTGAAAAGA TGATTGTCTC
 TTCACCACAC CAATTGATCA CCCTCCTCCA ACAAGCCAG AGAGACATAA
 GTTTTATTAG TTATTCTGAT CACTCTCAA GCCTTCTATA TAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACTTGCAT TGAACAAATTA ATAGAAAATAA
 CAGAAAAGTA GAATTCTAAA ATG

30 Still another example of such a DNA fragment ac-

cording to the invention is a DNA fragment which is identical with, derived from or comprises 5' flanking regions of the N23 gene with the sequence

	10	20	30	40	50	60	70
	<u>GAATTCTGAGCTGCCCGGGATCGATCCTCTAGAGTCGACCTGCAGCCCAGCTTGGATCAATCAATTAA</u>						
	<u>ECORI</u>						<u>Sall</u>
5	80	90	100	110	120	130	140
	TTCTATTGAGACACGATTGACAACAATTTTACATTATGAGACTATTTGGTTTTATTTGATCCAAA						
	150	160	170	180	190	200	210
	AAATTTAAGCTTAGATGATGATGAATTGAANNAATATTGTATTAAATNTGAAAAGTTNNNNNGGTTA						
	220	230	240	250	260	270	280
	ATGAATGCTATGATATTGATGGCTTGATNTATTNNCAGAATTGAAAGTATTAAAGAGAAGTGTAAAGAAA						
10	290	300	310	320	330	340	350
	AGAAAGTTAGCACACCAATAAGTATTGAGTTATTAATAACTTACATTCTTTCAAATGTTACATTG						
	360	370	380	390	400	410	420
	CATATAGAACATTATTGACAATCCTTATAACAGTTGCTACTGTTGAAAGACGTTCTTCAAAATTAAAATT						
	430	440	450	460	470	480	490
	ACTTAAATCATATCTAAATCAACAACTGTTACAAGATAGATTGAATGAGTTACTTATTTATCTATTGAA						
15	500	510	520	530	540	550	560
	AGTAAAGTGTAGAATTGTTGATTATAAAACTCTGATAAAATGATTGTTGAGTTAAACTAGAAAGAT						
	570	580	590	600	610	620	630
	TAATATAAAATTGATATTTATATAATATTAAGTCTTTAAATTCTGTAAAAAGACATTTTT						
	640	650	660	670	680	690	700
	AAATAATAAAATAAGCAACTCTTAATTAAATGAAACATCCCTTGTAAACCGAATCTCCATAATGT						
20	710	720	730	740	750	760	770
	AAAAATTAAATGCTTGATGAAAGTTTAATTGTTACTCAATAACTCAAAGGGTGTAAATATTTTTT						
	780	790	800	810	820	830	840
	TATCATTATATGTTGAAATATGAATGCACTAGTAATTAGTTAATGATAAAATATATTCTACAGATAT						
	850	860	870	880	890	900	910
	ATTTCTGCTCTGGCAACTCGTGAGAATTGAATATATTAAAGATGAAAGTCGTTACAATTTTTT						
25	920	930	940	950	960	970	980
	AGAATAAAATTTATATACAATTCTAGATTGTTATAAAACTCACATATTGTATGAGTATAAAACAT						
	990	1000	1010	1020	1030	1040	1050
	GAGCACACACCAAAACTAGTCAAATTAAAGTAAGGTGCTAATTATAGCGGCTAGCTAAGTAACCAAGTA						
	<u>DdeI</u>						

ATTAATG

The invention relates furthermore to any plasmid to be used when carrying out the method according to the invention and characterised by comprising a DNA fragment containing an inducible plant promoter 5 as herein defined. Particular examples of suitable plasmids according to the invention are pAR11, pAR29, pAR30, and N23-CAT, cf. Examples 3, 4, and 11. These plasmids allow recombination into the A. rhizogenes T DNA region.

10 The invention relates furthermore to any Agrobacterium strain to be used in connection with the invention and characterised by comprising a DNA fragment comprising an inducible plant promoter of root nodule-specific genes built into the T DNA 15 region and therefore capable of transforming the inducible promoter into plants. Particular examples of bacterium strains according to the invention are the A. rhizogenes strains AR1127 carrying pAR29, AR1134 carrying pAR30, AR1000 carrying pAR11, and 20 AR204-N23-CAT carrying N23-CAT.

It is obvious that the patent protection of the present invention is not limited by the embodiments stated above.

Thus the invention employs not exclusively 5' flanking regions of soybean leghemoglobin genes. It is well-known that the leghemoglobin genes of all leguminous plants have the same function, cf. Appleby (1974) in The Biology of Nitrogen Fixation, Quispel. A. Ed. North-Holland Publishing Company, 25 Amsterdam, Oxford, pages 499-554, and concerning the kidney bean PvLb1 gene it has furthermore been 30

proved that a high degree of homology exists with the sequences of the soybean Lbc_3 gene. It is also known that the expression of other root nodule-specific genes is regulated in a similar manner like the leghemoglobin genes. The invention includes thus the use of 5' flanking regions of leghemoglobin genes or other root nodule-specific genes of all plants in case the use of such DNA fragments makes the expression of a desired gene product the subject matter of the regulation characterised by the present invention.

The present invention allows also the use of such fragments of any origin which under natural conditions exert or mediate the regulation characterised by the present invention. The latter applies especially to such fragments which can be isolated from DNA fragments from gene libraries or genomes through hybridization with labelled sequences of 5' flanking regions of soybean leghemoglobin genes.

It is well-known that it is possible to alter nucleotide sequences of non-important sub-regions of 5' flanking regions without causing an alteration of the promoter activity and the regulation. It is also well-known that an alteration of sequences of important subregions of 5' flanking regions renders it possible to alter the binding affinities between nucleotide sequences and the factors or effector substances necessary or responsible for the transcription initiation and the translation initiation and consequently to improve the promoter activity and/or the regulation. The present invention includes, of course, also the use of DNA fragments

containing such altered sequences of 5' flanking regions, and in particular DNA fragments can be mentioned which have been produced by recombining sequences of 5' flanking regions of any gene with 5 5' flanking regions of root nodule-specific genes provided the use of such DNA fragments subjects the expression of a desired gene product to the regulation characterised by the present invention.

It should be noted that the transformation of micro-
10 organisms is carried out in a manner known per se,
cf. e.g. Maniatis et al., (1982), Molecular Cloning,
A Laboratory Manual, Cold Spring Harbor Laboratory.

The transformation of plant cells, i.e. introduction
of plasmid DNA into plant cells, is also carried
15 out in a manner known per se, cf. Zambryski et
al., (1983), EMBO J. 2, 2143-2150.

Cleavage with restriction endonucleases and di-
gestion with other DNA modifying enzymes are well-
known techniques and are carried out as recommended
20 by the suppliers.

The Agrobacterium rhizogenes 15834 rif^R was used
as a typical representative of A. rhizogenes: see
White et al., I.Bact., Vol. 141 (1980), 1134-1141.

Example 1

25 Sequence determination of 5' flanking regions of
soybean leghemoglobin genes

From a soybean gene library the four soybean leg-

hemoglobin genes Lba, Lbc₁, Lbc₂, and Lbc₃ are provided as described by Jensen, E.Ø. et al., Nature Vol. 291, No. 3817, 677-679 (1981). The genetically stable in-bred invariable soybean species "Glycine max.var.Evans" was used as a starting material for the isolation of the DNA used for the construction of said gene library. The 5' flanking regions of the four soybean leghemoglobin genes are isolated, as described by Jensen, E.Ø., Ph D Thesis, Institut for Molekylær Biologi, Århus Universitet (1985), and the DNA sequences determined by the use of the dideoxy method as described by Sanger, F., J. Mol. Bio. 143, 161-178 (1980) and indicated in the sequence scheme.

15 Example 2

Construction of Lbc₃-5'-3'-CAT

The construction has been carried out in a sequence of process steps as described below:

a) Sub-cloning the Lbc₃ gene

- 20 The Lbc₃ gene was isolated on a 12Kb EcoRI restriction fragment from a soybean DNA library, which has been described by Wiborg et al., in Nucl. Acids Res. (1982) 10, 3487. A section of the fragment is shown at the top of the attached Scheme 2. This
25 fragment was digested by the enzymes stated and then ligated to pBR322 as indicated at the Scheme. The resulting plasmids Lbc₃HH and Lbc₃HX were subsequently digested by PvuII and religated, which resulted in two plasmids called pLpHH and pLpHX.

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b) Sub-cloning 5' flanking sequences from the Lbc₃ gene

For this purpose pLpHH was used as shown in the attached Scheme 3. This plasmid was opened by means 5 of PvuII and treated with exonuclease Bal31. The reaction was stopped at various times and the shortened plasmids were ligated into fragments from pBR322. These fragments had been treated in advance as shown in Scheme 3, in such a manner that in one 10 end they had a DNA sequence TTC --- AAG ---.

After the ligation a digestion with EcoRI took place, and the fragments containing 5' flanking sequences were ligated into EcoRI digested pBR322. 15 These plasmids were transformed into E. coli K803, and the plasmids in the transformants were tested by sequence analysis. A plasmid, p213 5'Lb, isolated from one of the transformants, contained a 5' flanking sequence terminating 7 bp before the Lb ATG 20 start codon in such a manner that the sequence is start codon in such a manner that the sequence is as follows:

2Kb
-5' flanking --- AAAGTAGAATTC
Lbc₃ sequence

25 E.coli K803 is a typical representative of the E. coli K12 recipient strains.

c) Sub-cloning 3' flanking region of the Lbc₃ gene

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For this purpose pLpHX was used which was digested by XhoII. The ends were partially filled out and excess single-stranded DNA was removed with S1 nuclease, as shown in the attached Scheme 4. The 5 fragment shown was ligated into pBR322 which had been pretreated as shown in the Scheme. The construction was transformed into E. coli K803. One of the transformants contained a plasmid called Xho2a-3'Lb. As the XhoII recognition sequence is positioned immediately after the Lb stop codon, cf. Scheme 2, the plasmid contained about 900 bp of the 3' flanking region, and the sequence started with GAATTCTACAA---.

The construction of Lb promoter cassette

15 An EcoRI/SphI fragment from Xho2a-3'Lb was mixed with a BamHI/EcoRI fragment from p213-5'Lb. These two fragments were ligated via the BamHI/SphI cleavage sites into a pBR322 derivative where the EcoRI recognition sequence had been removed, cf. Scheme 20 4. The ligated plasmids were transformed into E. coli K803. A plasmid in one of the transformants contained the correct fragments, and it was called pEJLb 5'-3'-1.

Construction of the Lbc₃ 5'3'-CAT gene

25 The CAT gene of pBR322 was isolated on several smaller restriction fragments, as shown in the attached Scheme 5. The 5' coding region was isolated as an AluI fragment which was subsequently ligated into pBR322, treated as stated in the Scheme. This

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was transformed into E. coli K803. Several transformants contained the correct plasmid. One was taken out and called Alu11. The 3' coding region was isolated on a TaqI fragment. This fragment was
5 treated with exonuclease Bal31, whereafter EcoRI linkers were added. Then followed a digestion with EcoRI and a ligation to EcoRI digested pBR322. The latter was transformed into E. coli K803 and the transformants were analysed. A plasmid, Taq 12,
10 contained the 3' coding region of the CAT gene plus 23 bp 3' flanking sequences subsequently terminating in the following sequence CCCCGAATTG. Subsequently the following fragments were ligated together to EcoRI digested
15 pEJLb5'-3'-1: EcoRI/PvuII fragment from AluI, PvuII/DdeI fragment from pBR322 and DdeI/EcoRI fragment from Taq 12. This ligation mixture was transformed into E. coli K803. Several transformants contained the correct plasmid. One was taken out
20 and was called pEJLb 5'-3' CAT 15.

Example 3

a.

Cloning and integration of the soybean Lbc₃-5'-3'-CAT gene.

25 Two EcoRI fragments (No. 36 and No. 40) of the T_L-DNA region of A. rhizogenes 15834 pRI plasmid was used as "integration sites". Thus the Lbc₃-5'-3'-CAT gene was subcloned (as 3,6 Kb BamHI/SalI fragment) into two vectors pAR1 and pAR22 carrying the
30 above EcoRI fragments. The resulting plasmids pAR29

and pAR30 were separately mobilized into A. rhizogenes 15834 rif^R using a plasmid helper system; see E. van Haute et al. (1983), EMBO J. 3, 411-417. Neither pAR29 nor pAR30 can replicate in Agrobacterium. Therefore the selection by means of rifampicin 100 µg/ml and the plasmid markers spectinomycine 100 µg/ml, streptomycine 100 µg/ml or kanamycine 300 µg/ml will select A. rhizogenes bacteria having integrated the plasmids via homologous recombination through the EcoRI fragments 36 or 40. The structure of the resulting T_L-DNA regions - transferred to the transformed plant lines L5-9 and L6-23 - has been indicated at the bottom of the attached Scheme 6. In this Scheme is furthermore for the L6-23 line shown the EcoRI and HindIII fragments carrying the Lbc₃-5'-3'-CAT gene and therefore hybridizing to radioactively labelled Lbc₃-5'-3'-CAT DNA used as a probe, cf. Example 4a.

20bCloning and integration of the soybean Lbc₃ gene.

The EcoRI fragment No. 40 has here been used as "integration site". The Lbc₃ gene was therefore sub-cloned (as a 3,6 Kb BamHI fragment into the pAR1 vector and transferred into the T_L-DNA region as stated in a. The structure of the T_L-DNA region, transferred to the transformed plant line L8-35, has been shown at the bottom of the attached Scheme 7. This Scheme furthermore shows the EcoRI and HindIII fragments carrying the Lbc₃ gene and there-

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fore hybridizing with radioactively labelled Lbc_3
DNA used as a probe, cf. Example 4b.

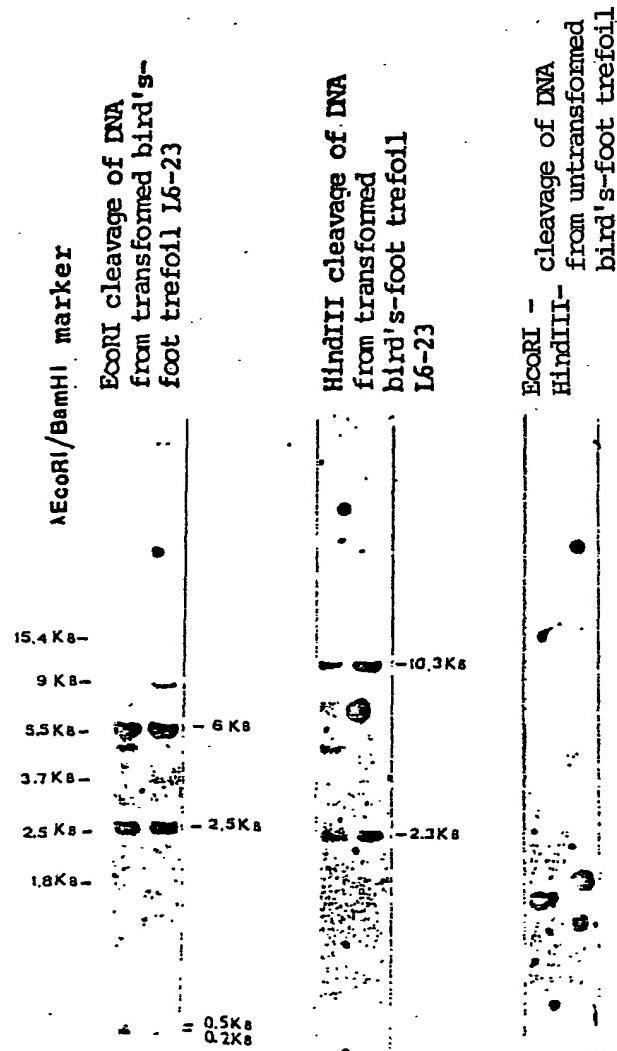
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Example 4.

a.

Demonstration of the soybean Lbc₃-5'-3'-CAT gene in transformed plants of bird's-foot trefoil.



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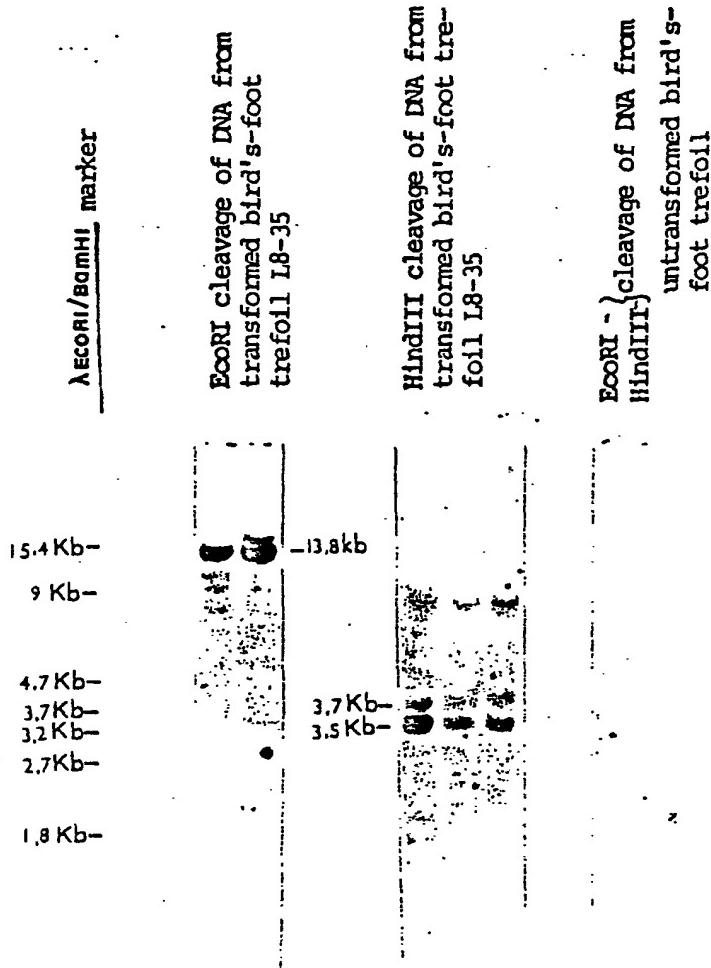
DNA extracted from transformed lines (L6-23) or untransformed control plants and cleaved by the restriction enzymes EcoRI and HindIII was analyzed by Southern-hybridization. Radioactively labelled 5 Lbc₃-5'-3'-CAT gene was used as a probe for demonstrating corresponding sequences in the transformed lines. The bands marked with numbers correspond to restriction fragments constituting parts of the Lbc₃-5'-3'-CAT gene as stated in the restriction 10 map (Scheme 6) of Example 3a.

b.

Demonstration of the soybean Lbc₃ gene of transformed plants of bird's-foot trefoil.

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 λ ECORI/BamHI marker

DNA extracted from transformed lines (L8-35) or untransformed control plants and cleaved by the restriction enzymes EcoRI and HindIII was analyzed by Southern-hybridization. Radioactive Lbc_3 gene was used as a probe for detecting corresponding sequences in the transformed lines. The bands marked with numbers correspond to restriction fragments constituting parts of the Lbc_3 gene as stated in the restriction map (Scheme 7) of Example 3b.

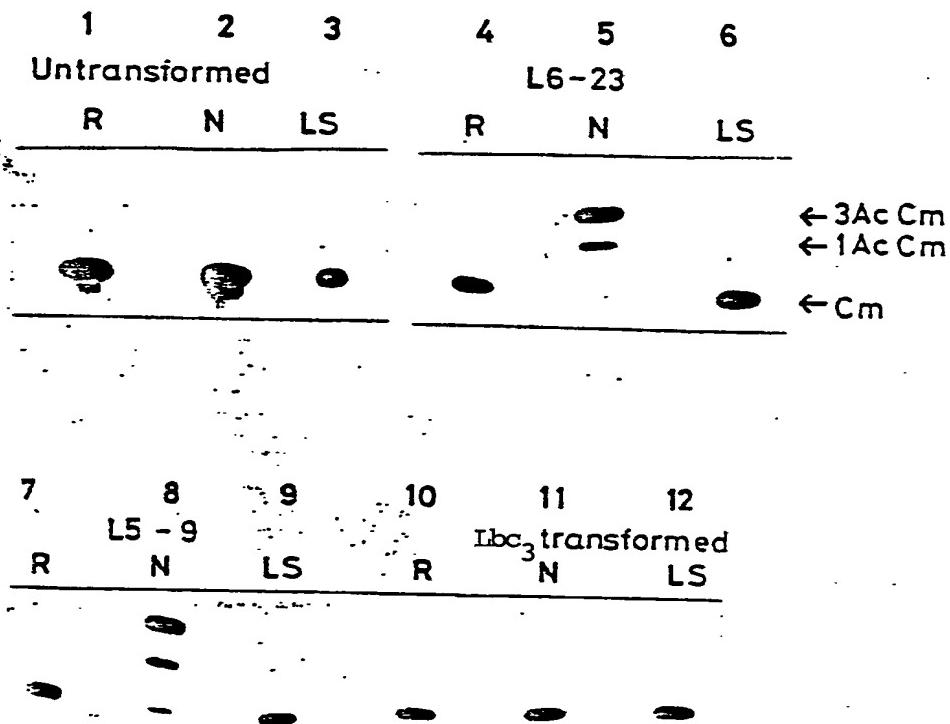
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Example 5

a.

Expression of the Lbc_3 -5'-3'-CAT gene in various tissues of bird's-foot trefoil.



The activity of the chloroamphenicol acetyl transferase (CAT) enzyme is measured as the amount of acetylated chloroamphenicol (AcGm) produced from ^{14}C -chloroamphenicol. In (a) the acetylated forms 5 1AcGm and 3AcGm appear, which have been separated from Gm through thin-layer chromatography in chloroform/methanol (95:5). The columns 1-3 show that no CAT activity occurs in root (R), nodule (N), as well as leaves + stem (LS) of untransformed plants 10 of bird's-foot trefoil. The columns 4-6 and 7-9 show the CAT activity in corresponding tissues of Lbc₃-5'-3'-CAT transformed L6-23 and L5-9 plants. The conversion of chloroamphenicol in columns 5 and 8 shows the organ-specific expression of the 15 Lbc₃-5'-3'-CAT gene in root nodules. The columns 10-12 show the lack of CAT activity in plants transformed with the Lbc₃ gene.

b.

Table

	L6-23		L5-9	
	CAT activity		CAT activity	
Root	0		0	
Nodule	68830 cpm/ μg protein.h		154,000 cpm/ μg protein.h	
Leaves +				
25 Stem	0		0	

In the Table (b) the CAT activity in Lbc₃-5'-3'-CAT transformed L5-9 and L6-23 plants has been stated as the amount of ^{14}C -chloroamphenicol converted into acetylated derivatives. The amount of radioactivity in the acetylated derivatives has been 30

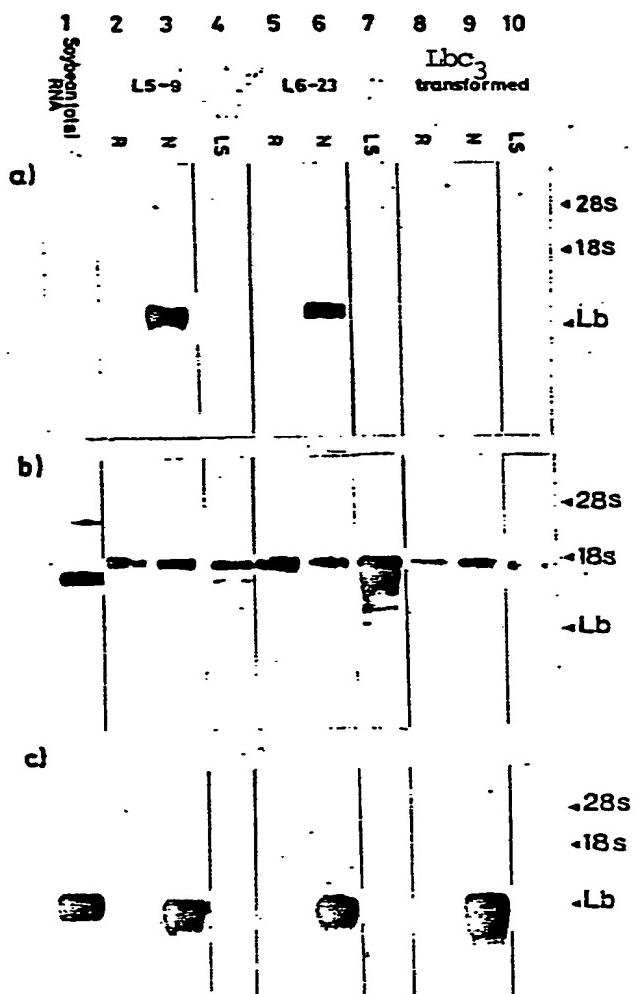
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counted by liquid scintillation and stated in cpm/ μ g protein · hour.

Example 6

Transcription test (Northern analysis) on tissues
5 of Lbc₃-5'-3'-CAT transformed and Lbc₃ transformed
Lotus plant lines.



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5 μ g of total RNA extracted from root (R), nodule (N) or leaves + stem (LS) and separated in formaldehyde agarose gels were transferred onto nitrocellulose. Column 1 contains 5 μ g of total RNA from 5 20-day-old soybean nodules as control plants. The columns 2-4 and 5-7 contain total RNA from root, nodule or leaves + stem, respectively, of the Lbc₃-5'-3'-CAT transformed lines L5-9 and L6-23. The columns 8-10 contain RNA from corresponding tissues 10 of bird's-foot trefoil transformed by means of A. rhizogenes carrying the Lbc₃ gene in the T_L-DNA. In (a) radioactive DNA of the CAT coding sequence has been used as a probe for hybridization. The organ-specific transcription of the Lbc₃-5'-3'-15 CAT gene in root nodules from the L5-9 and L6-23 lines appears from columns 3 and 6. In (b) the transcript for the constitutive ubiquitine gene(s) is visualized using a cDNA probe for the human ubiquitine gene for the hybridization. In (c) the 20 nodule-specific transcription of bird's-foot trefoil own leghemoglobin genes is shown. A cDNA probe of the Lba gene of soybean has been used for this hybridization.

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Example 7

Determination of the transcription initiation site
(CAP site) of the Lbc₃ promoter of soybean in trans-
formed root nodules of bird's-foot trefoil.



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The position of the "CAP site" was determined on the nucleotide level by means of primer extension. A synthetic oligonucleotide 5' CAACGGTGGTATATCCAGTG3' complementary to the nucleotides 15-34 in the coding sequence of the CAT gene was used as primer for the enzyme reverse transcriptase. As a result single-stranded cDNA was formed the length of which corresponds to the distance between the 5' end of the primer and the 5' end of the primed mRNA. A 83 nucleotide cDNA strand would be expected according to the knowledge of the transcription initiation site of soybean Lbc_3 gene. Columns 2, 3, and 4 from left to right show the produced DNA strands when the primer extension has been operated on polyA⁺-purified mRNA from transformed root nodules of bird's-foot trefoil, transformed leaves + stem of bird's-foot trefoil, and untransformed root nodules of bird's-foot trefoil, respectively. The 85, 86, 87, 88, and 90 nucleotides long cDNA strand shown in column 2 proved correctly Lbc_3 promoter function in bird's-foot trefoil. The CAP sites corresponding to the cDNA sequences generated are indicated with asterisks (*) on the partial sequence of the Lbc_3 5'3'-CAT region given. In the sequence the TATA box of the Lbc_3 promoter and the corresponding translation initiation codon of the CAT coding sequence are underlined.

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Example 8

Demonstration of the correct developmental control of the Lbc₃-5'-3'-CAT gene in transformed plants of bird's-foot trefoil (L6-23).

	Stage 1: No visible nodules	Stage 2: Emerging nodules	Stage 3: Distinct white nodules	Stage 4: Small pink nodules	Stage 5: Later stages of maturity
5 CAT activity in cpm/ μ g protein · hour	0	0	32.6	342.3	1255*
Nitrogenase activity nmol ethylene/ μ g protein · hour	0	0	0	0.5	2.7

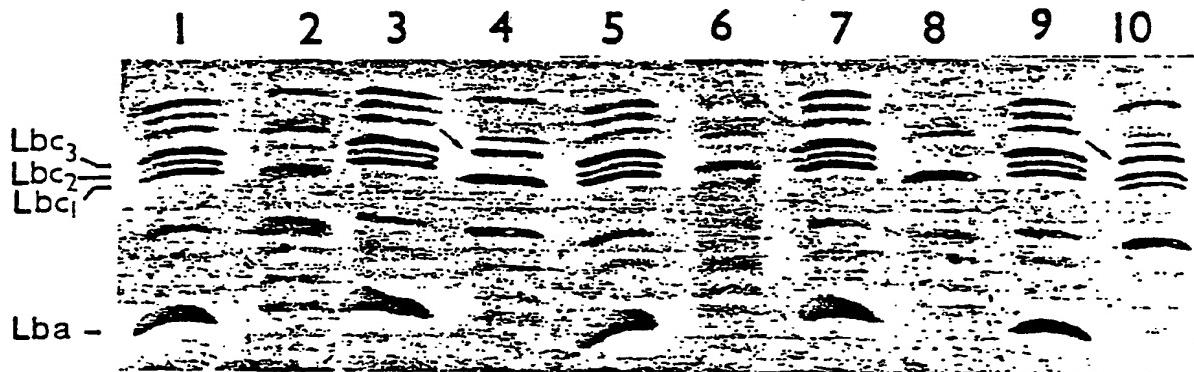
10 * Substrate limited reaction; actual activity about 68000 cpm/ μ g protein · hour.

Chloroamphenicol acetyl transferase and nitrogenase activity were measured on cut off pieces of root with nodules at the different developmental stages 15 indicated. The CAT activity can be detected in the white distinct nodules whereas the nitrogenase activity did not appear until the small pink nodules have developed. The latter development corresponds to the development known from soybean control plants 20 and described by Marcker et al. EMBO J. 1984, 3, 1691-95. The CAT activity was determined as in Example 5. The nitrogenase activity was measured

as acetylene reduction capacity of the nodules followed by gaschromatographic determination of ethylene.

Example 9

5 Demonstration of Lbc₃ protein in bird's-foot trefoil plants transformed with the soybean Lbc₃ gene.



Proteins extracted from root nodules of Lbc₃ transformed (L8-35), Lbc₃-5'-3'-CAT transformed and nontransformed plants were separated by isoelectric focussing at a pH gradient of 4 to 5. The columns 1, 3, 5, 7, and 9 show Lbc₁, Lbc₂, Lbc₃, and Lba proteins synthesized in soybean control root nodules. Column 2 shows proteins from root nodules of Lbc₃-5'-3'-CAT transformed L6-23-bird's-foot trefoil 15 plants, whereas the columns 6 and 8 show proteins from nontransformed plants. The columns 4 and 10 show soybean Lbc₃ protein synthesized in root nod-

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ules of bird's-foot trefoil plants (L8-35) transformed with the Lbc_3 gene. The Lbc_3 protein band is indicated by an arrow.

Example 10

5 Expression of the Lbc_3 -5'-3'-CAT gene requires the
5' Lbc_3 promoter region.

The Lbc_3 -5'-3'-CAT gene construction carries a 2 Kb 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 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2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220 2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280 2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340 2345 2350 2355 2360 2365 2370 2375 2380 2385 2390 2395 2400 2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460 2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520 2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580 2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640 2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700 2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760 2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820 2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880 2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940 2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000 3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060 3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120 3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540 3545 3550 3555 3560 3565 3570 3575 3580 3585 3590 3595 3600 3605 3610 3615 3620 3625 3630 3635 3640 3645 3650 3655 3660 3665 3670 3675 3680 3685 3690 3695 3700 3705 3710 3715 3720 3725 3730 3735 3740 3745 3750 3755 3760 3765 3770 3775 3780 3785 3790 3795 3800 3805 3810 3815 3820 3825 3830 3835 3840 3845 3850 3855 3860 3865 3870 3875 3880 3885 3890 3895 3900 3905 3910 3915 3920 3925 3930 3935 3940 3945 3950 3955 3960 3965 3970 3975 3980 3985 3990 3995 4000 4005 4010 4015 4020 4025 4030 4035 4040 4045 4050 4055 4060 4065 4070 4075 4080 4085 4090 4095 4100 4105 4110 4115 4120 4125 4130 4135 4140 4145 4150 4155 4160 4165 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5170 5175 5180 5185 5190 5195 5200 5205 5210 5215 5220 5225 5230 5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 6235 6240 6245 6250 6255 6260 6265 6270 6275 6280 6285 6290 6295 6300 6305 6310 6315 6320 6325 6330 6335 6340 6345 6350 6355 6360 6365 6370 6375 6380 6385 6390 6395 6400 6405 6410 6415 6420 6425 6430 6435 6440 6445 6450 6455 6460 6465 6470 6475 6480 6485 6490 6495 6500 6505 6510 6515 6520 6525 6530 6535 6540 6545 6550 6555 6560 6565 6570 6575 6580 6585 6590 6595 6600 6605 6610 6615 6620 6625 6630 6635 6640 6645 6650 6655 6660 6665 6670 6675 6680 6685 6690 6695 6700 6705 6710 6715 6720 6725 6730 6735 6740 6745 6750 6755 6760 6765 6770 6775 6780 6785 6790 6795 6800 6805 6810 6815 6820 6825 6830 6835 6840 6845 6850 6855 6860 6865 6870 6875 6880 6885 6890 6895 6900 6905 6910 6915 6920 6925 6930 6935 6940 6945 6950 6955 6960 6965 6970 6975 6980 6985 6990 6995 7000 7005 7010 7015 7020 7025 7030 7035 7040 7045 7050 7055 7060 7065 7070 7075 7080 7085 7090 7095 7100 7105 7110 7115 7120 7125 7130 7135 7140 7145 7150 7155 7160 7165 7170 7175 7180 7185 7190 7195 7200 7205 7210 7215 7220 7225 7230 7235 7240 7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 7775 7780 7785 7790 7795 7800 7805 7810 7815 7820 7825 7830 7835 7840 7845 7850 7855 7860 7865 7870 7875 7880 7885 7890 7895 7900 7905 7910 7915 7920 7925 7930 7935 7940 7945 7950 7955 7960 7965 7970 7975 7980 7985 7990 7995 8000 8005 8010 8015 8020 8025 8030 8035 8040 8045 8050 8055 8060 8065 8070 8075 8080 8085 8090 8095 8100 8105 8110 8115 8120 8125 8130 8135 8140 8145 8150 8155 8160 8165 8170 8175 8180 8185 8190 8195 8200 8205 8210 8215 8220 8225 8230 8235 8240 8245 8250 8255 8260 8265 8270 8275 8280 8285 8290 8295 8300 8305 8310 8315 8320 8325 8330 8335 8340 8345 8350 8355 8360 8365 8370 8375 8380 8385 8390 8395 8400 8405 8410 8415 8420 8425 8430 8435 8440 8445 8450 8455 8460 8465 8470 8475 8480 8485 8490 8495 8500 8505 8510 8515 8520 8525 8530 8535 8540 8545 8550 8555 8560 8565 8570 8575 8580 8585 8590 8595 8600 8605 8610 8615 8620 8625 8630 8635 8640 8645 8650 8655 8660 8665 8670 8675 8680 8685 8690 8695 8700 8705 8710 8715 8720 8725 8730 8735 8740 8745 8750 8755 8760 8765 8770 8775 8780 8785 8790 8795 8800 8805 8810 8815 8820 8825 8830 8835 8840 8845 8850 8855 8860 8865 8870 8875 8880 8885 8890 8895 8900 8905 8910 8915 8920 8925 8930 8935 8940 8945 8950 8955 8960 8965 8970 8975 8980 8985 8990 8995 9000 9005 9010 9015 9020 9025 9030 9035 9040 9045 9050 9055 9060 9065 9070 9075 9080 9085 9090 9095 9100 9105 9110 9115 9120 9125 9130 9135 9140 9145 9150 9155 9160 9165 9170 9175 9180 9185 9190 9195 9200 9205 9210 9215 9220 9225 9230 9235 9240 9245 9250 9255 9260 9265 9270 9275 9280 9285 9290 9295 9300 9305 9310 9315 9320 9325 9330 9335 9340 9345 9350 9355 9360 9365 9370 9375 9380 9385 9390 9395 9400 9405 9410 9415 9420 9425 9430 9435 9440 9445 9450 9455 9460 9465 9470 9475 9480 9485 9490 9495 9500 9505 9510 9515 9520 9525 9530 9535 9540 9545 9550 9555 9560 9565 9570 9575 9580 9585 9590 9595 9600 9605 9610 9615 9620 9625 9630 9635 9640 9645 9650 9655 9660 9665 9670 9675 9680 9685 9690 9695 9700 9705 9710 9715 9720 9725 9730 9735 9740 9745 9750 9755 9760 9765 9770 9775 9780 9785 9790 9795 9800 9805 9810 9815 9820 9825 9830 9835 9840 9845 9850 9855 9860 9865 9870 9875 9880 9885 9890 9895 9900 9905 9910 9915 9920 9925 9930 9935 9940 9945 9950 9955 9960 9965 9970 9975 9980 9985 9990 9995 9999

The Lbc_3 -5'-3'-CAT gene construction was opened in 15 the unique XbaI site shown above, and digested with the exonuclease Bal31. A SalI linker fragment was ligated onto the blunt ends generated and the shortened SalI fragments carrying the Lbc_3 -5'-3'-CAT gene were transferred into *L. corniculatus*. The effect 20 of removing promoter sequences was measured as CAT activity. End points of the deleted 5' region are given as the distance from the CAP site in nucleotides.

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	5'Lbc ₃	3'Lbc ₃	CAT activity Cpm/ μ g protein/hrs.		
			Root	Nodule	Leaf
2000	-	CAT	0	80000	0
-950	-	-	0	10000	0
-474	-	-	0	3000	0
-230	-	-	0	3000	0
-78	-	-	0	0	0

5 The drastically reduced level of CAT activity expressed from the Lbc₃ promoter deleted to nucleotide -230 and the zero activity from the promoter deleted to nucleotide -78 demonstrates that the Lbc₃ promoter region is required for the root nodule specific expression of the Lbc₃-5'-3'-CAT gene.

Example 11

Construction of the N23-CAT gene.

The N23 gene was isolated from a soybean DNA library as described in the enclosed paper of Sandal, Bojsen 15 and Marcker. The N23-CAT gene was constructed from the modified Lbc₃-5'-3'-CAT gene carried on plasmid pEJ5'-3'-CAT101 as described in the Applicant's copending application No. 86 11 4704.9 concerning "Expression of Genes in Yeast", and a 1 Kb. EcoRI, 20 DdeI fragment containing the N23 5' promoter region. The position of the EcoRI and DdeI sites in the N23 promoter region is indicated on the DNA sequence shown below. The cloning procedure used is outlined

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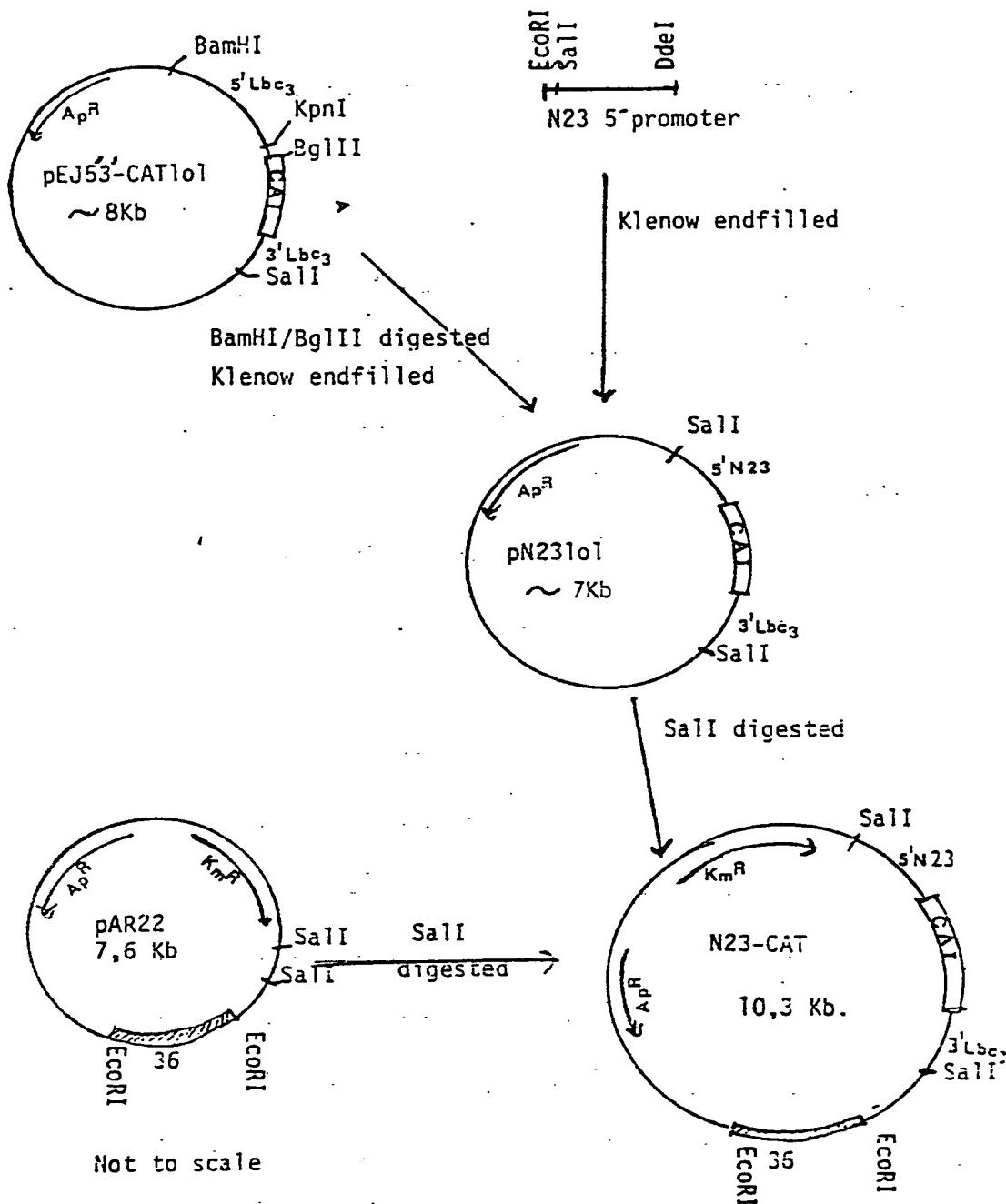
56

below. The disclosure of the papers of Sandal et al., the EP application, and the paper of Jensen et al., Nature 321 (12 June 1986), 669-674, including the references cited should be considered incorporated into the present description as a means to amend, illustrate, and clarify it.

The N23-CAT gene was transferred to plants by the same method as the Lbc₃-5'-3'-CAT gene.

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DNA sequence of the 5'-promotor region from the
N23 gene

10 20 30 40 50 60 70
GAATTCGAGCTCGCCGGGGATCGATCCCTAGAGTCGACCTGCAGCCCCAAGCTTGGATCAATCAATTAA
EcoRI
 Sali

5 80 90 100 110 120 130 140
TTCTATTGAGACACGATTGAACAATTTACATTATGAGACTATTTGGTTTTTATTTGATCCAAA

15 150 160 170 180 190 200 210
AAATTAAAGCTTAGATGATGAATTGAANNAATATTGTATTAATNNNTGAAAAGTTNNNNNGTTA

20 220 230 240 250 260 270 280
ATGAATGCTATGATATTGATGGCTTGTATNTATTNNCAGAATTGAAAGTATTAAAGAGAACTGTTAAGAAA

10 290 300 310 320 330 340 350
AGAAGTTAGCACACCAATAGAAGTATTGAGTTATTAACCTTAAACTTTAGATTCTTTCAATTGTTACATTG

15 360 370 380 390 400 410 420
CATATAGAATTATTGACAATCCTTATAACAGTTGCTACTGTTGAAAGACGTTCTTCAAAATTAAAATT

20 430 440 450 460 470 480 490
ACTTAAATCATATCTAAATCAACAATGTTACAAGATAGATTGAATGAGTTAGTTATTTTCTATTGAA

15 500 510 520 530 540 550 560
AGTAAAGTGTAGAATTGTTGATTATAAAACTCTGATAAAATGATTTGCAGTTAAAAAAACTAGAACAGAT

20 570 580 590 600 610 620 630
TAATATAAAAATTGATATTATATAATATATAAGTCTCTTAAATTCTGTAAAAAAGACATTTT

25 640 650 660 670 680 690 700
AAATAATAAAATAAGCACTCTTAATTAAATGAAACATCCCTTGTTAACCGAATCTCCATAATG

30 710 720 730 740 750 760 770
AAAAATTAAATGCTGATGGAAGTTTTAATTGTTCTACTCAATACTCAAAGGGTTGTAATATTTTT

35 780 790 800 810 820 830 840
TATCATTTATATGTTGTAATATGAATGCACTAGTAATTAGTTAATGATAAAATATATTCTACAGATAT

40 850 860 870 880 890 900 910
ATTCTGCTCTGGCAACTCGTGAGAATTGAATATATTATAAGATGAAAGGTCGTTACAATTTTT

45 920 930 940 950 960 970 980
AGAATAAAATATTATATAACATTCTAGATTGTTATAAAATTACACATATTGTATGAGTATAAAATACAT

50 990 1000 1010 1020 1030 1040 1050
GAGCACACACAAACTAGTCTCAATTAAAGTAAGGTGCTAATTATTAGCGGCTAGCTAAGTAACCAAGTA
BglI

ATTAATG

Example 12

Organ-specific expression of the soybean N23-CAT gene in root nodules of *L.corniculatus* and *Trifolium repens*.

5 The activity of chloroamphenicol acetyl transferase (CAT) was measured as in example 5 and is given in cpm/ μ g protein/hrs.

Table a.

10	N23-CAT transformed <i>L.corniculatus</i>	CAT activity	
		Untransformed	<i>L.corniculatus</i>
Root nodule	86150	0	
Root	0	0	

Table b.

15	N23-CAT transformed <i>T.repens</i>	CAT activity	
		Untransformed	<i>T.repens</i>
Root nodule	148000	0	
Root	0	0	

Table (a) and b) shows the organ-specific expression of the N23-CAT gene in root nodules of *L.corniculatus* and *T.repens*. *L.corniculatus* was inoculated with *Rhizobium loti*, while *T.repens* was inoculated with *Rhizobium trifolii*.

In connection with the invention it has thus been proved that root nodule-specific genes can be expressed organ-specifically upon transfer to other plants, here *Lotus corniculatus* and *Trifolium re-*

pens. It has furthermore been proved that the 5' flanking regions comprising the promoter are controlled by the organ-specific regulatory mechanism as the organ-specific control of the Lbc_3 -5'-3'-CAT gene in Lotus corniculatus took place at the transcription level. The Lbc_3 -5'-3'-CAT gene transferred was thus only transcribed in root nodules of transformed plants and not in other organs such as roots, stems, and leaves.

10 The expression of the Lbc_3 -5'-3'-CAT gene in root nodules of transformed plants also followed the developmental timing known from soybean root nodules. No CAT activity could be detected in roots or small white root nodules (Example 8). A low
15 activity was present in the further developed white distinct nodules, whereas a high activity could be measured in the small pink nodules and mature nodules developed later on.

The organ-specific expression and the correct development of transferred root nodule-specific genes, here exemplified by the Lbc_3 -5'-3'-CAT gene, allows as a particular use a functional expression of root nodule-specific genes also in other plants beyond leguminous plants. When all
25 the root nodule-specific plant genes necessary for the formation of root nodules are transferred from a leguminous plant to a non-root-nodule-forming plant species, the correct organ-specific expression proved above allows production of functionally
30 active, nitrogen-fixing root nodules on this plant upon infection by Rhizobium. In this manner these plants can grow without the supply of external

inorganic or organic nitrogen compounds. Root nodule-specific promoters, here exemplified by the Lbc₃ and N23 promoters, must be used in the present case for regulating the expression of the transferred genes.

According to the present invention a root nodule-specific promoter is used for expressing genes. The gene product or function of the gene product improves the function of the root nodule, e.g. by altering the oxygen transport, the metabolism, the nitrogen fixation or the nitrogen absorption.

Root nodules are thus used for the synthesis of biological products improving the plant per se or which can be extracted from the plant later on. A root nodule-specific promoter can be used for expressing a gene. The gene product or compound formed by said gene product constitute the desired product(s).

In connection with the present invention it has furthermore been proved that the soybean Lbc₃ leg-hemoglobin protein per se, i.e. the Lbc₃ gene product, is present in a high concentration in root nodules of bird's-foot trefoil plants expressing the Lbc₃ code sequence under the control of the Lbc₃ promoter. The latter has been proved by cloning the genomic Lbc₃ gene of the soybean into the integration vector pAR1, said genomic Lbc₃ gene containing the coding sequence, the intervening sequences, and the 5' and 3' flanking sequences. A 3.6 Kb BamHI fragment Lbc₃HH, cf. Example 2, was cloned into the pAR1 plasmid and transferred to

bird's-foot trefoil as stated previously.

The high level of Lbc_3 protein, cf. Example 9, found in transformed root nodules of bird's-foot trefoil and corresponding to the level in soybean 5 root nodules proves an efficient transcription of the Lbc_3 promoter and an efficient processing and translation of Lbc_3 mRNA in bird's-foot trefoil.

The high level of the CAT activity present in transformed root nodules is also a result of an efficient 10 translation of mRNA formed from the chimeric Lbc_3 gene. The leader sequence on the Lbc_3 gene is decisive for the translation initiation and must determine the final translation efficiency. This 15 efficiency is of importance for an efficient synthesis of gene products in plants or plant cells. An Lbc_3 or another leghemoglobin leader sequence can thus be used for increasing the final expression level of a predetermined plant promoter. The construction of a DNA fragment comprising a Lb leader 20 sequence as first sequence and an arbitrary promoter as second sequence is a particular use of the invention when the construction is transferred and expressed in plants.

During nodule development around 30 different plant 25 encoded polypeptides (nodulins) are specifically synthesized. Apart from the leghemoglobins, nodulins include nodule-specific forms of uricase (Bergmann et al (1983) EMBO. J. 2, 2333-2339), glutamine synthetase (Cullimore et al (1984) J.Mol. 30 Appl. Genetics 2, 589-599) and sucrose synthase (Morell and C peland (1985) Plant. Physiol. 78,

149-154). The function of most nodulins are, however, at present unknown.

Many nodulin genes have nevertheless been isolated and characterised during the last five years. These 5 include nodulins from several different legumes. Examples of such isolations and characterisations are widespread in the literature such as (Fuller et al (1983) Proc. Natl. Acad. Sci. 80, 2594-2598), (Sengupta-Gopalan et al (1986) Molec. Genet. 10 203, 410-420), (Bisseling et al (1985) in Proceedings of the 6th Int. symp. on Nitrogen Fixation, Martinus Nijhoff Publishers pp 53-59.), and (Gebhardt et al (1986) EMBO J. 5, 1429-1435). All of these genes contain nodule-specific regulatory 15 sequences. Such sequences and in fact entire 5' flanking regions and 3' flanking regions can furthermore be synthesized by automated oligonucleotide synthesis knowing the DNA sequences for the Lbc₃ and N23 genes given in this description. Entire 20 nodule-specific genes can also be isolated with known recombinant techniques as described in the above papers and by (Maniatis et al (1982) Molecular cloning. A Laboratory Manual, Cold Spring Harbour Laboratory, New York).

25 The described method to obtain nodule-specific expression of genes can thus be reconstructed and performed according to the invention by any one skilled in the art of molecular genetics.

The method to obtain nodule-specific expression is 30 not dependent on the A. rhizogenes plant transformation described. Any other plant transformation

system e.g. A. tumefaciens systems, direct gene transfer or microinjection can equally be applied.

The A. rhizogenes system has been used and characterised by a number of scientific groups and is thus well-known from the literature. The characteristics of the system is described in:

- Willmitzer et al. (1982), Molec.Gen. Genet. 186, 16-22,
- Chilton et al. (1982), Nature 295, 432-434,
- 10 Simpson et al. (1986), Plant.Molec.Biol. 6, 493-415,
- Tepfer D. (1983), Molecular Genetics of the Bacteria - Plant interaction,
Springer Verlag, Berlin Heidelberg pp
15 248-258,
- White and Nester (1980), J.Bact. 144, 710-720,
- Jaynes and Strobel (1981), Int.Rev. of Cytol. Sup. 13, 105-125,
- 20 White and Nester (1980), J. Bact. 141, 1134-1141,
- Pomponi et al. (1983), Plasmid 10, 119-129, and

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Slightom et al. (1986), J. Biol. Chem.
261, 108-121.

The latter two publications describe the restriction map and nucleotide sequence of the A. rhizogenes 5T_L-DNA segment used in the transformation system described here. With this information it is possible to anybody skilled in molecular genetics to use and reconstruct the "intermediate vectors" and the A. rhizogenes strains described here.

Claims:

1. A method of expressing genes in plants, parts of plants, and plant cell cultures by introducing into a cell thereof a recombinant DNA segment containing both the gene to be expressed and a 5' flanking region comprising a promoter sequence, and optionally a 3' flanking region, and culturing of the transformed cells in a growth medium, characterised by using as the recombinant DNA segment a DNA fragment comprising an inducible plant promoter (as defined) from root nodule-specific genes.
2. A method as claimed in claim 1, characterised by using a DNA fragment comprising an inducible plant promoter (as defined) and being identical with, derived from or comprising 5' flanking regions of root nodule-specific genes.
3. A method as claimed in claim 2, characterised by using a DNA fragment comprising an inducible plant promoter (as defined) and being identical with, derived from or comprising 5' flanking regions of root nodule-specific genes, said DNA fragment causing an expression of a gene which is induced in root nodules at specific stages of development and as a step of the symbiosis, whereby nitrogen fixation occurs.
4. A method as claimed in claims 1-3 for the expression of root nodule-specific genes, characterised by using a DNA fragment comprising an inducible plant promoter (as defined)

from root nodule-specific genes.

5. A method as claimed in claims 1-3 for the expression of genes in leguminous plants, parts of leguminous plants, and leguminous plant cell cultures, characterised by using a DNA fragment comprising an inducible plant promoter (as defined) from root nodule-specific genes.

6. A method as claimed in claims 1-5, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of leghemoglobin genes.

7. A method as claimed in claim 6, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of soybean leghemoglobin genes.

8. A method as claimed in claim 7, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of the Lba gene with the sequence

GAGATAACATT ATAATAATCT CTCTAGTGTC TATTATTAT TTTATCTGGT
GATATATACC TTCTCGTATA CTGTTATT TTCAATCTTG TAGATTACT
25 TCCTTTATT TTATAAAAAA GACTTTATT TTAAAGGTGA
ATTTGAAAAA CATGCTCTT GACAATTTC TGTTCCCTT TTCATCATGG
GGTTAAATCT CATAGTGCT CTATTCAATA ATTTGGGCTC AATTTAATTA
GTAGAGTCTA CATAAAATT ACCTTAATAG TAGAGAATAG AGAGTCCTGG
AAAGTTGGTT TTTCTCGAGG AAGAAAGGAA ATGTTAAAAAA CTGTGATATT
TTTTTTTGTT ATTAAATAGTT ATGTTTATAT GAAAAGTGA AATAAAATAAA
30 CTAACCATAT TAAATTAGA ACAACACTTC AATTATTTT TTAATTGAT
TAATTTAAAAA ATTATTTGAT TAAATTTTT AAAAGATCGT TGTTCTTCT
TCATCATGCT GATTGACACC CTCCACAAGC CAAGAGAAAC ACATAAGCTT
TGTTTTGTC ACTCTCCAAG CCCTCTATAT AAACAAATAT TGGAGTGAAAG

TTGTTGCATA ACTTGCATCG AACAAATTAAAT AGAAAATAACA GAAAATCAA
AAAGAAATAT G.

9. A method as claimed in claim 7, characterised by the DNA fragment comprising 5 the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of the Lbc₁ gene with the sequence:

TTCTCTTAAT ACAATGGAGT TTTTGTGAA CATAACATACA TTTAAAAAAA
AATCTCTAGT GTCTATTAC CCGGTGAGAA GCCTTCTCGT GTTTTACACA
10 CTTAATATT ATTATATCCT CAACCCCCACA AAAAGAATA CTGTTATATC
TTTCCAAACC TGTAGATTAA TTTATTATT TATTATTTT TACAAAGGAG
ACTTCAGAAA AGTAATTACA TAAAGATAGT GAACATCATT TTATTATTAA
TAATAAACTT TAAAATCAA CTTTTTATA TTTTTGTTA CCCTTTCAT
TATTGGGTGA AATCTCATAG TGAAGCCATT AAATAATTG GGCTCAAGTT
TTATTAGTAA AGTCTGCATG AAATTAACT TAACAATAGA GAGAGTTTTC
15 GAAAGGGAGC GAATGTTAAA AAGTGTGATA TTATATTAA TTTCGATTAA
TAATTATGTT TACATGAAAA CATAACAAAA AATACTTTA AATTCAAGAAT
AATACTTAAA ATATTATTAA GCTTAATTGA TTAACTGAAA ATTATTTGAT
TAGGATTTG AAAAGATCAT TGGCTCTTCG TCATGCCGAT TGACACCCCTC
CACAAAGCCAA GAGAAACTTA AGTTGTAAC AC TTTCTCACTC CAAGCCTCT
ATATAAAACAT GTATTGGATG TGAAGTTATT GCATAACTTG CATGAAACAA
TAGAAAATAA CAAAAAAAAG TAAAAAGTA GAAAAGAAAT ATG,

20 10. A method as claimed in claim 7, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of the Lbc₂ gene with the sequence:

25 TCGAGTTTT ACTGAACATA CATTATTAA AAAAAACTCT CTAGTGTCCA
TTTATTCGGC GAGAACCTT CTCGTGCTT ACACACTTTA ATATTATTAT
ATCCCCACCC CCACCAAAAA AAAAAAACCT GTTATATCTT TCCRGTCAT
TTATTCTTA TTTTACAAA GGAAACTTCA CGAAAGTAAT TACAAAAAAG
ATAGTGAACA TCATTTTTT AGTTAAGATG AATTTTAAAA TCACACTTTT
TTATTTTT TTGTTACCTT TTTCATTATT GGGTGAAATC TCATAGTGAA
ACTATTAAAT AGTTGGGCT CAAGTTTAT TAGTAAAGTC TGCAATGAAAT
30 TTAACCTTAAT AATAGAGAGA GTTTTGAAA GGTAAACGAAT GTTAGAAAGT
GTGATATTAT TATAGTTTA TTTAGATTAA TAATTATGTT TACATGAAAA
TTGACAATT TTGTTAAAT TTCAAGACTAA TACTTAAATT ACTTATTAC
TTTAAGATT TGAAAAGATC ATTTGGCTCT TCATCATGCC GATTGACACC
CTCCACAAAGC CAAGAGAAAC TTAAGTTGTA ATTTTCTAA CTCCAAGCCT
TCTATATAAA CACGTATTGG ATGTGAAGTT GTGCATAAC TTGCATTGAA
CAATAGAAAT AACAAACAAAG AAAATAAGTG AAAAAAGAAA TATG,

11. A method as claimed in claim 7, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions 5 of the *Lbc₃* gene with the sequence:

TATGAAGATT AAAAATACA CTCATATATA TGCCATAAGA ACCAACAAAA
 10 GTACTATTTA AGAAAAGAAA AAAAAAAACCT GCTACATAAT TTCCAATCTT
 GTAGATTTAT TTCTTTATT TTATATAAGG AGAGTTAAAA AAATTACAAA
 ATAAAAATAG TGAACATCGT CTAAGCATT TTATATAAGA TGAATTAA
 AAATATAATT TTTTGTCATA AACGTATGT ATCTTGTCTT AGAGCCATT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTTCCT CCGAGTTGA
 TATAAAAAAA ATTGTTCCC TTTGATTAT TGGATAAAAT CTCGTAGTGA
 CATTATATTA AAAAATTAG GGCTCAATT TTATTAGTAT AGTTGCATA
 AATTTAACT TAAAATAGA GAAAATCTGG AAAAGGGACT GTTAAAAAGT
 GTGATATTAG AAATTTGTCG GATATATTAA TATTTTATT TATATGGAAA
 CTAAAAAAAT ATATATTAAA ATTAAATT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTTGAAAAGA TGATTGTCTC
 15 TTCACCATAAC CAATTGATCA CCCTCCTCCA ACAAGCCAAG AGAGACATAA
 GTTTTATTAG TTATTCTGAT CACTCTTCAA GCCTTCATAA TAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACTTGCAT TGAACAAATTA ATAGAAATAA
 CAGAAAAGTA GAAAAGAAAT ATG.

12. A method as claimed in claim 7, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of the *Lbc₃-5'-3'-CAT* gene with the sequence:

TATGAAGATT AAAAATACA CTCATATATA TGCCATAAGA ACCAACAAAA
 25 GTACTATTTA AGAAAAGAAA AAAAAAAACCT GCTACATAAT TTCCAATCTT
 GTAGATTTAT TTCTTTATT TTATATAAGG AGAGTTAAAA AAATTACAAA
 ATAAAAATAG TGAACATCGT CTAAGCATT TTATATAAGA TGAATTAA
 AAATATAATT TTTTGTCATA AACGTATGT ATCTTGTCTT AGAGCCATT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTTCCT CCGAGTTGA
 TATAAAAAAA ATTGTTCCC TTTGATTAT TGGATAAAAT CTCGTAGTGA
 30 CATTATATTA AAAAATTAG GGCTCAATT TTATTAGTAT AGTTGCATA
 AATTTAACT TAAAATAGA GAAAATCTGG AAAAGGGACT GTTAAAAAGT
 GTGATATTAG AAATTTGTCG GATATATTAA TATTTTATT TATATGGAAA
 CTAAAAAAAT ATATATTAAA ATTAAATT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTTGAAAAGA TGATTGTCTC
 TTCACCATAAC CAATTGATCA CCCTCCTCCA ACAAGCCAAG AGAGACATAA
 GTTTTATTAG TTATTCTGAT CACTCTTCAA GCCTTCATAA TAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACTTGCAT TGAACAAATTA ATAGAAATAA
 CAGAAAAGTA GAATTCTAAA ATG

13. A method as claimed in claim 5, characterised by the DNA fragment comprising the inducible plant promoter and being identical with, derived from or comprising 5' flanking regions of 5 the N23 gene with the sequence:

10 20 30 40 50 60 70
GAATTTCAGCTCGCCGGGATCGATCCCTAGAGTCGACCTGCAGCCCAGCTTGGATCAATCAATTAA
EcoRI
Sall

80 90 100 110 120 130 140
TTCTATTGAGACACGATTGAAACATTACATTATGAGACTATTTTGTTTTATTTGATCCAAAA

150 160 170 180 190 200 210
10 AAATTTAAAGCTTAAAGATGATGATGAAATTGAANNAATTGTATAATNNNTGAAAAGTTNNNNNGTTA

220 230 240 250 260 270 280
ATGAATGCTATGATATTGATGGCTTGATNTATTNNCAGAATTGAAAGTATTAAGAGAAGTGTAAAGAAA

290 300 310 320 330 340 350
AGAAGTTAGCACACCAATAGAAGTATTGAGTTATTAACCTTAAACTTAACTTAACTTAACTTACATTG

360 370 380 390 400 410 420
15 CATATAGAATTTTATTGACAATCCTTATAACAGTTGCTACTGTTGAAAGACGTTCTCAAAATTAAAAATT

430 440 450 460 470 480 490
ACTTAAATCATATCTAAACATCAACAATGTTACAAGATAGATTGAATGAGTTAGTTATTTATCTATTGAA

500 510 520 530 540 550 560
AGTAAAGTGTAGAATTGTTGATTATAAAACTCTGATAAAATGATTTGCAAGTTAAAAAAACTAGAAGAT

570 580 590 600 610 620 630
20 TAATATAAAATTGATATTTATATAATATAAGTCTCTTAAATTCTGTAAAAAAAGACATTTT

640 650 660 670 680 690 700
AAATAATAAAATAAGCAACTCTTAATTTTAATGAAACATCCCTTGTAAACCGAATCTCCATAATGT

710 720 730 740 750 760 770
AAAAAAATTAAATGCTTGATGGAAGTTTTAATTGTTCTACTCAAAACTCAAAGGGTTGTAAATATTTTTT

780 790 800 810 820 830 840
25 TATCATTATGTTGAAATATGAATGCACTAGTAATTAGTTAATGATAAAATATATTCTACAGATAT

850 860 870 880 890 900 910
ATTCTGTCCTGGCAACTCGTGAGAATTGAATATATAAAGATGAAAGCTGTTACAATTTTTTT

920 930 940 950 960 970 980
AGAATAAAATTTATATACAAATTCTAGATTGTTATAAAATTCAACATATTGTATGAGTATAAAATACAT

990 1000 1010 1020 1030 1040 1050
30 GAGCACACACCAAAACTAGTCTCAAATTAAAGTAAGGTGCTAATTATTAGCGGCTAGCTAACCAAGTA
Ddel

ATTAATG

14. A method as claimed in any of the claims 1-13, characterised by the 3' flanking region of the genes to be expressed being a 3' flanking region of root nodule-specific genes of 5 any origin.

15. A method as claimed in claim 14, characterised by the 3' flanking region being of leghemoglobin genes.

16. A method as claimed in claim 14, characterised by the 3' flanking region being of soybean leghemoglobin genes.

17. A method as claimed in claim 16, characterised by the 3' flanking region being of the Lba, Lbc₁, Lbc₂ or Lbc₃ gene with the following sequences, respectively:

Lba

1590 1620
TAA TTA GTA TCT ATT GCA GTA AAG TGT AAT AAA TAA ATC TTG

1650 1680
20 TTT CAC TAT AAA ACT TGT TAC TAT TAG ACA AGG GCC TGA TAC AAA ATG TTG GTT AAA ATA

1710 1740
ATG GAA TTA TAT AGT ATT GGA TAA AAA TCT TAA GGT TAA TAT TCT ATA TTT GCG TAG GTT

1770 1800
TAT GCT TGT GAA TCA TTA TCG GTA TTT TTC CTT TCT GAT AAT TAA TCG GTA AAT TA

1830 1860
25 ACA AAT ARG TTC AAA ATG ATT TAT ATG TTT CAA AAT TAT TTT AAC AGC AGG TAA AAT GTT

ATT TGG TAC GAA AGC TAA TTC GTC GA

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Lbc₁

1320
TAA/TT AGG ATC TAC TGC ATT GCC GTA

AAG TGT AAT AAA TAA ATC TTG TTT CAA CTA AAA CTT GTT ATT AAA CAA GTT CCC TAT ATA
1350 1380

AAT GTT GTT TAA AAT AAG TAA ATT TCA TTG TAT TGG ATA AAC ACT TTT AAG TTA TAT ATT
1410 1440

5 TCC ATA TAT TTA CGT TTG TGA ATC ATA ATC GAT ACT TTA TAA AAA TAA ATT CCA AAT AAT
1470 1500

TTA TAC GTT TTA AAA ATT ATT TT

Lbc₂

10 TAG/GAT CTA CTA TTG CCG TCA AGT
1140

GTA ATA AAT AAA TTT TGT TTC ACT AAA ACT TGT TAT TAA ACA AGT CCC CGA TAT ATA AAT
1170 1200

CTT GGT TAA AAT AAG TAA ATT ATA CGG TAT TGA TAA ACA ATC TTA AGT TTT ATA TAT AGT
1230 1260

TCC ATA TAC TAA AGT TTG TGA ATC ATA ATC GA
1290

15 and Lbc₃

TAG/GAT CTA CAA TTG CCT TAA AGT GTC ATA AAT AAA
990 1020

TAT TAT TTC ACT AAA ACT TGT TAT TAA ACC AAG TTC TCG ATA TAA ATG TTG GTT AAA CTA
1050 1080

20 AGT AAA TTA TAT GGT ATT GGA TAA ACA ATC TTA AGC TT
1110

18. A method as claimed in claim 1 of preparing
a polypeptide by introducing into a cell of a plant,
a part of a plant or a plant cell culture a recombi-
25 nant plasmid, characterised by using
as the recombinant plasmid a plasmid comprising an
inducible plant promoter (as defined) of root nod-
ule-specific genes.

19. A DNA fragment comprising an inducible plant promoter (as defined) to be used when carrying out the method as claimed in claims 1-18, characterised by being identical with, derived from or comprising a 5' flanking region of root nodule-specific genes of any origin.

20. A DNA fragment as claimed in claim 19, characterised by being identical with, derived from or comprising a 5' flanking region of plant leghemoglobin genes.

21. A DNA fragment as claimed in claim 20, characterised by being identical with, derived from or comprising a 5' flanking region of soybean leghemoglobin genes.

15 22. A DNA fragment as claimed in claim 21, characterised by being identical with, derived from or comprising a 5' flanking region of the Lba gene with the sequence:

GAGATACATT ATAATAATCT CTCTAGTGTC TATTTATTAT TTTATCTGGT
20 GATATATACC TTCTCGTATA CTGTTATTT TTCAATCTTG TAGATTTACT
TCTTTATTT TTATAAAAAA GACTTTATTT TTTTAAAAAA AATAAAGTGA
ATTTTAAAAA CATGCTCTTT GACAATTTC TGTTCCCTTT TTCATCATTG
GGTTAAATCT CATAGTGCCT CTATTCAATA ATTTGGGCTC AATTTAATTA
GTAGAGTCTA CATAAAATTT ACCTTAATAG TAGAGAATAG AGAGTCTTGG
AAAGTTGGTT TTTCTCGAGG AAGAAAGGAA ATGTTAAAAA CTGTGATATT
TTTTTTTG G ATTAATAGTT ATGTTTATAT GAAAAGTGA AATAAATAAA
25 CTAACCATAT TAAATTAGA ACAACACTTC AATTATTTT TTAATTGAT
TAATTAAAAA ATTATTTGAT TAAATTTTT AAAAGATCGT TGTTCTTCT
TCATCATGCT GATTGACACC CTCCACAAAGC CAAGAGAAC ACATAAGCTT
TGGTTTCTC ACTCTCCAAG CCCTCTATAT AAAACAAATAT TGGAGTGAAG
TTGTTGCATA ACTTGCATCG AACAAATTAAT AGAAAATAACA GAAAATTAAG
AAAGAAATAT G,

23. A DNA fragment as claimed in claim 21,
 characterised by being identical with,
 derived from or comprising a 5' flanking region of
 the Lbc₁ gene with the sequence:

TTCTCTTAAT ACAATGGAGT TTTTGTGAA CATAACATACA TTTAAAAAAA
 5 AATCTCTAGT GTCTATTTAC CCGGTGAGAA GCCTTCTCGT GTTTTACACA
 CTTAATATT ATTATATCCT CAACCCCCACA AAAAAGAATA CTGTTATATC
 TTTCCAAACC TGAGATTAA TTTATTATT TATTTATTAA TACAAAGGAG
 ACTTCAGAAA AGTAATTACA TAAAGATAGT GAACATCATT TTATTTATTA
 TAATAAAACTT TAAAATCAA CTTTTTATA TTTTTGTTA CCCTTTCAT
 TATTGGGTGA AATCTCATAG TGAAGCCATT AAATAATTG GGCTCAAGTT
 TTATTAGTAA AGTCTGCATG AAATTTAAGT TAACAATAGA GAGAGTTTC
 10 GAAAAGGGAGC GAATGTTAAA AAGTGTGATA TTATTTTTA TTTCGATTAA
 TAATTATGTT TACATGAAAA CATAACAAAA AATACTTTA AATTCAAGAAT
 AATACTTAAA ATATTATTG GCTTAATTGA TTAACTGAAA ATTATTTGAT
 TAGGATTTTG AAAAGATCAT TGGCTCTTCG TCATGCCGAT TGACACCCCTC
 CACAAGCCAA GAGAAACTTA AGTTGTAAC AC TTCTCACTC CAAGCCTTCT
 ATATAAACAT GTATTGGATG TGAAGTTATT GCATAACTTG CATTGAACAA
 TAGAAAATAA CAAAAAAAG TAAAAAAAGTA GAAAAGAAAT ATG,

15 24. A DNA fragment as claimed in claim 21,
 characterised by being identical with,
 derived from or comprising a 5' flanking region of
 the Lbc₂ gene with the sequence:

TCGAGTTTT ACTGAACATA CATTTATTAA AAAAAGACTCT CTAGTGTCCA
 TTTATTCGGC GAGAACCTT CTCGTGCTT ACACACTTTA ATATTATTAT
 20 ATCCCCACCC CCACCAAAAA AAAAAGACT GTTATATCTT TCCAGTACAT
 TTATTTCTTA TTTTACAAA GGAAACTTCA CGAAAGTAAT TACAAAAAAG
 ATAGTGAACA TCATTTTTT AGTTAAGATG AATTTAAAAA TCACACTTTT
 TTATTTTTT TTGTTACCTT TTTCATTATT GGGTGAATC TCATAGTGA
 ACTATTAAAT AGTTGGGCT CAAGTTTAT TAGTAAAGTC TGCACTGAAAT
 TTAACTTAAAT AATAGAGAGA GTTTGGAAA GGTAAACGAAT GTTAGAAAGT
 GTGATATTAT TATAGTTTA TTTAGATTAA TAATTATGTT TACATGAAAA
 TTGACAATT TTGTTAAAT TTCAGAGTAA TACTTAAATT ACTTATTTAC
 25 TTTAAGATT TGAAAAGATC ATTTGGCTCT TCATCATGCC GATTGACACC
 CTCCACAAAGC CAAGAGAAAC TTAAGTTGTA ATTTTCTAA CTCCAGCCT
 TCTATATAAA CACGTATTGG ATGTGAAGTT GTTGCATAAC TTGCATTGAA
 CAATGAAAT AACAAACAAAG AAAATAAGTG AAAAAGAAA TATG,

25. A DNA fragment as claimed in claim 21,
 characterised by being identical with,
 30 derived from or comprising a 5' flanking region of

the *Lbc₃* gene with the sequence:

TATGAAGATT AAAAATACCA CTCATATATA TGCCATAAGA ACCAACAAAA
 GTACTATTTA AGAAAAGAAA AAAAAAACCT GCTACATAAT TTCCAAATCTT
 GTAGATTTAT TTCTTTATT TTATATAAAGG AGAGTTAAAA AAATTACAAA
 5 ATAAAAATAG TGAACATCGT CTAAGCATTT TTATATAAGA TGAATTAA
 AAATATAATT TTTTGTCTA AATCGTATGT ATCTTGTCTT AGAGCCATTT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTTCCT CCGAGTTGA
 TATAAAAAAA ATTGTTCCC TTTTGATTAT TGGATAAAAT CTCGTAGTGA
 CATTATATTA AAAAATTAG GGCTCAATT TTATTTAGTAT AGTTGCATA
 AATTTTAACT TAAAATAGA GAAAATCTGG AAAAGGGACT GTTAAAAAGT
 GTGATATTAG AAATTGTCG GATATATTAA TATTTTATT TATATGGAAA
 10 CTAAAAAAAT ATATATTAA ATTAAATT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTGAAAAGA TGATTGTCTC
 TTCACCACAC CAATTGATCA CCCTCCTCCA ACAAGCCAAG AGAGACATAA
 GTTTTATTAG TTATTCTGAT CACTCTTCAA GCCTTCTATA TAAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACCTGCAT TGAACAATTAA ATAGAAATAA
 CAGAAAAGTA GAAAAGAAAT ATG.

15 26. A DNA fragment as claimed in claim 21,
 characterised by the DNA fragment
 comprising the inducible plant promoter being iden-
 tical with, derived from or comprising 5' flanking
 regions of *Lbc₃-5'-3'-CAT* gene with the sequence:

20 TATGAAGATT AAAAATACCA CTCATATATA TGCCATAAGA ACCAACAAAA
 GTACTATTTA AGAAAAGAAA AAAAAAACCT GCTACATAAT TTCCAAATCTT
 GTAGATTTAT TTCTTTATT TTATATAAAGG AGAGTTAAAA AAATTACAAA
 ATAAAAATAG TGAACATCGT CTAAGCATTT TTATATAAGA TGAATTAA
 AAATATAATT TTTTGTCTA AATCGTATGT ATCTTGTCTT AGAGCCATTT
 TTGTTAAAT TGGATAAGAT CACACTATAA AGTTCTTCCT CCGAGTTGA
 TATAAAAAAA ATTGTTCCC TTTTGATTAT TGGATAAAAT CTCGTAGTGA
 25 CATTATATTA AAAAATTAG GGCTCAATT TTATTTAGTAT AGTTGCATA
 AATTTTAACT TAAAATAGA GAAAATCTGG AAAAGGGACT GTTAAAAAGT
 GTGATATTAG AAATTGTCG GATATATTAA TATTTTATT TATATGGAAA
 CTAAAAAAAT ATATATTAA ATTAAATT CAGAATAATA CTTAAATTAT
 TTATTTACTG AAAATGAGTT GATTTAAGTT TTGAAAAGA TGATTGTCTC
 TTCACCACAC CAATTGATCA CCCTCCTCCA ACAAGCCAAG AGAGACATAA
 GTTTTATTAG TTATTCTGAT CACTCTTCAA GCCTTCTATA TAAATAAGTA
 TTGGATGTGA AGTTGTTGCA TAACCTGCAT TGAACAATTAA ATAGAAATAA
 30 CAGAAAAGTA GAATTCTAA ATG

27. A DNA fragment as claimed in claim 19,
 characterised by being identical with,

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derived from or comprising 5' flanking regions of
the N23 gene with the sequence:

GAATTGGAGCTGCCCGGGATCGATCCCTAGAGTCGACCTGCAGCCCAAGCTGGATCAATCAATTAA
5' ECORI SalI

5 TTCTATTGAGACACGATTGAAACAATTTCACATTATGAGACTATTTGGTTTTATTGTATCaaaa

150 160 170 180 190 200 210
AAATTTAAGCTTATGATGATGAATTGAANNAATATTGTATTAATNNNTGAAAAGTTNNNNNGTTA

220 230 240 250 260 270 280
ATGAATGCTATGATATTGATGGCTTGATNTATTNNCAGAATTGAAAGTATTAAAGAGAAGTGTARGAAA

10 AGAAGTTAGCACACCAATAAGAAGTATTGAGTTATTAATTTAAACTTAGATTCTTTCAAAATGTTACATTG

360 370 380 390 400 410 420
CATATAGAATTATTGACAATCCTTATAACAGTTGCTACTGTTGAAGACGTTCTTCAAAATTAAAATT

430 440 450 460 470 480 490
ACTTAAATCATATCTAAAATCAACATGTTACAAGATAGATTGAATGAGTTAGTTATTTATCTATTGAA

15 500 510 520 530 540 550 560
15 AGTAAAGTGTAGAATTGTTGATTATAAAACTCTGATAAAATGATTTGCAGTTAAAAAAACTAGAAAGAT

570 580 590 600 610 620 630
TAATATAAAATTGATATTATATAATATATAAGTCTCTTAAATTCTTGTAAAAAGACATTTT

640 650 660 670 680 690 700
AAATAATAAAATAAGCAACTCTTAATTAAATGAAACATCCCTTGTAAACCGAATCTCCATAATGT

20 710 720 730 740 750 760 770
20 AAAAATTAATGCTTGATGGAAGTTAAATTGTTACTCAATACTCAAAGGGTTGTAATATTTTTT

780 790 800 810 820 830 840
TATCATTATATGTTGAAATATGAATGCACTAGTAATTGTTAATGATAAAATATATTCTACAGATAT

850 860 870 880 890 900 910
ATTTCGTCTTGGCAACTCGTGAGAATTGAATATATTATAAGATGAAAGGTGTTACATTTTTT

25 920 930 940 950 960 970 980
25 AGAATAATATTATATAACATTCTAGATTGTTATAAAATTCAACATATTGTATGAGTATAAATACAT

990 1000 1010 1020 1030 1040 1050
GAGCACACACCAAACTAGTCTCAAATTAAAGTAAGGTGCTAATTATTAGCGGCTAGCTAAGTAACCAAGTA
Ddel

ATTAATG

28. A plasmid which can be used when carrying

out the method as claimed in claims 1-18,
characterised by comprising a DNA
fragment as claimed in any of the claims 19-27.

29. A plasmid as claimed in claim 28, characterised by being pAR29.

30. A plasmid as claimed in claim 28, characterised by being pAR30.

31. A plasmid as claimed in claim 28, characterised by being pAR11.

10 32. A plasmid as claimed in claim 28, characterised by being N23-CAT.

33. A transformant Agrobacterium rhizogenes 15834-strain which can be used when carrying out the method as claimed in any of the claims 1 to 18,
15 characterised by the bacterium strain being transformed by a plasmid according to any of the preceding claims 28 to 32.

34. A transformant Agrobacterium rhizogenes 15834-strain which can be used when carrying out the method as claimed in any of the claims 1 to 18,
20 characterised by the bacterium strain being transformed by pAR29 and being named AR1127.

35. A transformant Agrobacterium rhizogenes 15834-strain which can be used when carrying out the method as claimed in any of the claims 1 to 18,
25 characterised by the bacterium strain being transformed by pAR30 and being named AR1134.

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36. A transformant Agrobacterium rhizogenes 15834-strain which can be used when carrying out the method as claimed in any of the claims 1 to 18, characterised by the bacterium strain 5 being transformed by pAR11 and being named AR1000.

37. A transformant Agrobacterium rhizogenes 15834-strain which can be used when carrying out the method as claimed in any of the claims 1 to 18, characterised by the bacterium strain 10 being transformed by N23-CAT and being named AR204-N23-CAT.

38. Plants, parts of plants and plant cells, particularly of the family Leguminosae, obtainable by transformation with a recombinant DNA segment, 15 fragment or plasmid according to any one of the claims 1 to 37.